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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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3/15/07

MEMORANDUM

SUBJECT: PC 079401: Endosulfan – Developmental Neurotoxicity Feeding Study in Rats with Endosulfan [MRID# 46968301].

FROM: David G Anderson, Ph.D., Toxicologist
and
Judy Facey, Ph.D., Toxicologist
RRB-2, HED (7509P)

TO: Tracy Perry
SRRD (7508P)

THROUGH: Mary Elissa Reaves, Ph.D., Toxicologist
RRB-2, HED (7509P)

Bayer CropScience submitted a Developmental Neurotoxicity Feeding Study in the Rat with Endosulfan [MRID# 46968301] for the Endosulfan Task Force [ETF].

Summary: In a developmental neurotoxicity study (MRID 46968301), endosulfan (99.1% a.i, batch# EGPC400349)] was administered to 30 female Wistar Crl:WI (Han) rats per group in the diet at dose levels of 0, 50,150, or 400 ppm or 0, 3.74, 10.8 or 29.8 mg/kg bw/day from gestation day 6 through postnatal day 21.

The NOAEL for dams is 10.8 mg/kg/day. The LOAEL is 29.8 mg/kg/day based on decrease body weight, food consumption and food efficiency.

There was no NOAEL for pups. The LOAEL was the LDT at 3.74 mg/kg/day based on decreased pup weight on PND 11 and decreased weight gain at PND 4-11. At the MDT, possible delayed preputial separation in males occurred. No neurotoxic effects were seen at the LDT or MDT. Possible effects were shown at the HDT for PND 21 male rearing in the FOB [within historical control range], PND 21 male fixed perfused brains and a 0.158 mm statistically significant decrease in female morphometric measurements on the PND 21 hippocampal gyrus at the HDT, the only dose level measured [control 1.623 mm, HDT 1.469 mm, $p<0.05$], but these values in control and at the HDT were within historical control range of 1.38 to 1.69 mm. These possible neurotoxic related effects could be biologically significant and treatment related at higher doses, but are not definitively shown in this study at the HDT.

This study is classified ACCEPTABLE and satisfies the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, 83-6); OECD 426 (draft).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Rec'd in RRC 3/20/07

EPA Reviewer: David Anderson, Ph.D. & Judy Facey, Ph.D. **Signature:** WD Hazel, for,
RRB-2, Health Effects Division (7509P) **Date:** 3/15/07
EPA Secondary Reviewer: Elissa Reaves, Ph.D. **Signature:** WD Hazel, for
RRB-2, Health Effects Division (7509P) **Date:** 3/15/07

Template version 02/06

TXR#: 0054486

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (83-6); OECD 426 (draft)

PC CODE: 075401 **DP BARCODE: D327215**

TEST MATERIAL (PURITY): Endosulfan [99.1% pure][6,7,8,9,10,10-hexachloro-1,5,5a,9,9a-hexahydro-6,9-methano-2,4,3,-benzodioxathiepin-3-oxide]

CITATION: Gilmore, R.; Sheets, L.; Hoss, H. (2006) A Developmental Neurotoxicity Study with Technical Grade Endosulfan in Wistar Rats. Project Number: 201563, 05/D72/YF. Dated September 26, 2006. Unpublished study prepared by Bayer Corp. 1062 p. MRID# 46968301

SPONSOR: Endosulfan Task Force

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46968301), endosulfan (99.1% a.i, batch# EGPC400349) was administered to 30 female Wistar Crl:WI (Han) rats per group in the diet at dose levels of 0, 50, 150, or 400 ppm or 0, 3.74, 10.8 or 29.8 mg/kg bw/day from gestation day 6 through postnatal day 21; doses were adjusted to nearly constant mg/kg/day throughout lactation. Dams were evaluated for body weight, food consumption, clinical observations, and FOB on GD 13 and 20. Offspring were dosed only through nursing mothers and when eating the mother's diet. Subsets of dams with acceptable litters were evaluated for FOB on LD 11 and 21. Selected offspring from each group were evaluated using detailed clinical observations, body weight, food consumption, developmental landmarks for sexual maturation, automated measurements of activity in a figure eight maze, auditory startle habituation, learning and memory [passive avoidance and a water maze task]. Ophthalmic examinations were conducted. Tissues were collected for morphometry and microscopic examination on PND 21 [brain] and at study termination [brain, an assortment of neural tissue, and skeletal muscle]. Sperm analysis was performed on testes and epididymal sperm.

Body weight of dams was statistically significantly decreased at all dose levels on gestational day [GD] 13 and 20 and lactational day [LD] 0, 4 and 7 at the two highest dose levels. However, when this body weight decrease was correlated with food consumption data, food efficiency calculations suggested that a decreased food consumption from an unpalatable diet may have caused the body weight decrement at the LDT and the MDT. This was more certain at the LDT, but less definitive at the MDT. Only the HDT induced adverse body weight decrement when combined with food efficiency. No other effects were noted in dams. It is noted that a 90-day neurotoxicity study [MRID 46444401] in Wistar rats did not show body weight decrement in non-pregnant females at the HDT of 45.5 mg/kg/day. However, food consumption in these females

was decreased statistically at the 16.6 and 45.5 mg/kg/day during the first week of the 90-day study.

The NOAEL for dams is 3.7 mg/kg/day. The LOAEL is 10.8 mg/kg/day based on decrease body weight, food consumption and food efficiency.

Pup weight on a litter basis was decreased in males and females [males:8.3% - 13.2%, $p<0.01$; females: 8.0%-13.7%, $p< 0.01$] in all dose levels on PND 11 and PND 17 [males: 6.9%-8.8%, $p<0.05$] in males only. The male and female pup weight decrement at all dose levels at PND 11 was reflected in a decrement in the pup weight gain from PND 4-11. Although statistically significance occurred in data on male weight gain PND 11-17, this pup weight gain decrement is believed to be due failure to regain weight lost during the decrement from PND 4-11. No pup weight decrement was seen at birth or PND 4 at the LDT. Since it is unknown whether the pup weight decrement was due to unpalatable endosulfan in the mother's milk, a reduced milk supply or a toxic effect of endosulfan in the milk, it will be assumed to be a toxic effect until data are submitted to show otherwise.

Sexual development was delayed as shown by preputial separation in males at the MDT and HDT of [47.1 and 46.8 days, respectively compared with 44.9 days in controls] and delayed vaginal opening in females at the LDT [34.2 days] and MDT [34.2 days] and at HDT [34.0 days] compared with control at 33.0 days. The delayed preputial separation in males had no effect on sperm parameters measured at termination. The vaginal opening may have been incidental since the data were not dose related and within historical control range [32.0 to 34.6 days]. There was no difference in body weight among dosed groups and controls at the time of measurement.

No definitive effects were seen in the FOB with offspring. However, 1/16 and 3/16 PND 4 male pups and 0/16 and 2/16 PND 4 females showed minimal resistance to removal with minimal vocalization at the MDT and HDT, respectively. Other groups tested at PND 11, 21, 35 or 60 showed this effect in 0/16 offspring tested. PND 21 male pups showed dose related slight increased rearing [$p<0.05$] at the HDT and PND 21 female pups showed increased rearing at the MDT [6.4, $p<0.05$] and HDT [5.5, $p>0.05$] only.

No effects were shown when tested for acoustical startle, learning and memory in the passive avoidance or water maze tests, which showed habituation.

Perfused fixed brain weight in PND 21 male pups was reduced 5% at the HDT, but the body weight ratio was nominally increased. Brain weight for fixed perfused PND 21 and PND 75 females was unchanged as well as fixed perfused and non-perfused PND 75 male brain weight. Morphometric analysis of the female brains showed a decrease in hippocampal gyrus of 0.158 mm [a 10% decrease, $p<0.05$ compared with controls] at the HDT only.

There was no NOAEL for pups. The LOAEL was the LDT at 3.74 mg/kg/day based on decreased pup weight at PND 11 and decreased weight gain at PND 4-11. At the MDT, possible delayed preputial separation in males occurred. No neurotoxic effects were seen at the LDT or MDT. Possible effects were shown at the HDT for PND 21 male rearing in the FOB [within historical control range], PND 21 male fixed perfused brains and a 0.158 mm statistically significant decrease in female morphometric measurements on the PND 21 hippocampal gyrus at the HDT, the only dose level measured [control 1.623 mm, HDT 1.469 mm, $p<0.05$], but these values in control and at the HDT were within historical control range of 1.38 to 1.69 mm. These possible neurotoxicity-related effects could be biologically significant and treatment related at higher doses, but are not definitively shown in this study at the HDT.

This study is classified as **Acceptable/Non-guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats [OPPTS 870.6300, §83-6; OECD 426 (draft)] due to the pending review of the positive control data.

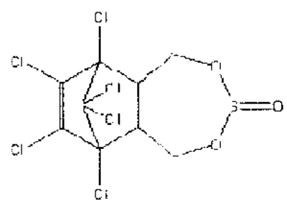
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:**	Endosulfan [6,7,8,9,10,10-hexachloro-1,5,5a,9,9a-hexahydro-6,9-methano-2,4,3,-benzodioxathiepin-3-oxide]
Description:	Light yellow tablets
Lot/batch #:	EGPC400349
Purity:	99.1 % a.i.
Compound stability:	Stable at room temperature
CAS # of TGAI:	115-29-7

Structure:



2. **Vehicle and/or positive control** None

3. **Test animals (P):** Test material was dissolved in acetone, mixed into the food and allowed to evaporate

Species:	Rat
Strain:	Wistar Crl:WI(Han)
Age at study initiation:	At least 15 wks for males and 12 wks for females
Wt. at study initiation:	females 159.2 – 218.9g
Source:	Charles River Laboratories, Raleigh, NC
Housing:	Suspended stainless steel cages during cohousing and suspended plastic cages with cage board bedding for gestation and lactation
Diet:	Purina Mills Rodent Diet 5002 in meal form *ad libitum*
Water:	Kansas City Municipal water *ad libitum*
Environmental conditions:	Temperature: 18-26° C Humidity: 30-70% Air changes: 10 /hr/day Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	5/2/05 [receipt]- 5/9/05 [release for study]

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: 5/9/2005; End: 8/23/2005.

2. **Study schedule:** The maternal animals [P-generation] were mated and assigned to the study as they were determined to be sperm positive. The test material was administered via the feed from gestation day 6 through 21 of lactation/postnatal development. On postnatal day [PND] 21, pups were weaned and maternal animals sacrificed at study termination on PND 75 [±5 days].

3. **Mating procedure:** Females were paired 1:1 with males of the same strain and source. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm was detected in a vaginal smear was designated gestation day 0. After successful mating, each pregnant female was placed into an individual cage with a solid bottom and bedding, where it was maintained through gestation and lactation.

4. **Animal assignment**: Mated females were assigned as they became pregnant to dose groups as indicated in Table 1. Dams were assigned to functional observation testing as shown.

   Offspring were assigned to testing subgroups at the time of litter standardization on postnatal day 4 (Table 1). Details of the assignment of offspring to the various test groups were copied as an image below from the report.

   [In addition to the information copied with regard to offspring testing groups, the remaining pages on Study Design and Procedures through page 21 of the current DER were copied from pages 18-34, 63-64 of the submitted report.]

Offspring were assigned to testing subgroups at the time of litter standardization on postnatal day 4 (Table 1). An animal allocation program written in SAS [1] was used to assign offspring to the following four sets (designated A-D) for assessment at each age. One male and/or female per litter (approximately 16 (minimum 10)/sex/dietary level, representing at least 20 litters per level): Motor activity (Set A), Auditory Startle (Set B), Passive Avoidance, Water Maze and Functional Observational Battery (Set C). On PND21, the whole brain was collected from a separate group of randomly selected offspring (Set D; 10/sex/dietary level; representing 20 litters per level) for micropathologic examination and morphometric analysis. The remaining pups assigned to Set D (—10/sex/dietary level) were reserved for possible use as replacement animals or were otherwise sacrificed on PND21 without necropsy examination.

At approximately 50-60 days of age, randomly selected animals (a minimum of 10/sex/dietary level, representing at least 20 litters per level) from Sets A, B and C were subjected to an ophthalmologic examination. At termination (PND75 (± 5 days)), these animals were anesthetized and sacrificed by perfusion, with neural and muscle tissues collected for microscopic examination. At termination on PND75 (±5 days), brains were collected from additional randomly selected animals (10/sex/dietary group; representing 20 litters per level). These brains were weighed (fresh tissue weight) and then discarded.

The remaining animals assigned to sets A-C were sacrificed without routine gross necropsy examination or collection of tissues.

Table1. Study Design[a]

Experimental Parameter	Dietary Level (mg/kg/day)			
	Control	3.74	10.8	29.8
Maternal Animals				
No. of maternal animals assigned	30	30	30	30
FOB (GD 13, 20/ LD 11, 21)	30/ 10	30/ 10	30/ 10	30/ 10
Offspring				
Detailed clinical observation/ FOB (PND4, 11,21, 35(±1),45(±1), 60(±1)	16 (min. 10)/sex	16 (min. 10)/sex	16 (min. 10)/sex	16 (min. 10)/ sex
Motor activity (PND 13,17,21,60(±2)	15-16 (min. 10)/sex	15-16 (min. 10)/sex	15-16 (min. 10)/sex	15-16 (min. 10)/sex
Auditory startle habituation (PND 22,60 (±2)	15-16 (min. 10)/sex	15-16 (min. 10)/sex	15-16 (min. 10)/sex	15-16 (min. 10)/sex
Learning and memory (PND 22/ 29, 60 (±2)/ 67 (±2)	16 (min. 10)/sex	16 (min. 10)/sex	16 (min. 10)/sex	16 (min. 10)/sex
Brain weight PND 21 PND 75 (± 5)	 10/ sex 10/ sex	 10/ sex 10/ sex	 10/ sex 10/ sex	 10/ sex 10/ sex
Neuropathology PND 21 PND 75 (± 5)	 10/ sex 10/ sex	 10/ sex 10/ sex	 10/ sex 10/ sex	 10/ sex 10/ sex

a data obtain from pg 20 study report MRID 46968301

The method of animal assignment minimized potential problems related to litter effects, by using at least one pup/litter. For FOB and motor activity testing, the same individual animals were evaluated at all scheduled time points. For the selection of animals and testing paradigms for cognitive (learning and memory) assessment, the same animals were used for assessments at the weanling and adult ages, but different tests were used at the two ages.

5. **Dose selection rationale:** The rationale for dose selection was based on the results of a multi-generation reproduction study [5], a 90-day toxicity study in Sprague-Dawley rats [61, a subchronic neurotoxicity study in Wistar rats [71 and a pilot study with pregnant Wistar rats [8] to guide selection of the highest dietary level. In the reproduction study, technical-grade endosulfan was administered via the diet at nominal concentrations of 0, 3, 15 and 75 ppm, beginning 12 weeks prior to co-housing (pre-mating phase). At 15 ppm, there were no compound-related findings in the P-generation or the Fl offspring. At 75 ppm, decreased body weight gain and increased liver and kidney weights in P(0) and F(1) males, and significant decrease in body weight gain during the first week of study (67% of control) was evident. No reproductive effects were seen at any dietary level. In the first 90-day study, endosulfan was administered to Sprague-Dawley rats at nominal concentrations of 0, 10, 30, 60 and 360 ppm [6]. Compound-related effects at the highest dietary level included decreased body weight gain in mates (slight) and females (32%), with decreased food consumption (12%) in high-dose females during the first two weeks of exposure. There was an increased incidence of hair loss in females at the 60 and 360 ppm dietary levels, particularly at the beginning of the study. Lower plasma and erythrocyte cholinesterase activities were evident in high-dose females, while brain cholinesterase activity was increased at study termination in females at the 60 and 360 ppm dietary levels. Liver and kidney weight was increased at the high dose in both sexes and at 60 ppm in males. In the subchronic neurotoxicity study, endosulfan was administered to Wistar rats via the diet at nominal concentrations of 0, 40, 225 and 600 ppm [7]. The only clinical sign involved one high-dose female that convulsed during the first week of treatment, and was found dead on study day 51. There were statistically significant decreases in food consumption in high- dose males and in females at the mid and high

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dose for the first two weeks of the study. Mid- and high-dose females also had decreased body weight (maximum 5-7%) and decreased plasma cholinesterase (ChE) activity (approximately 50% at each level), however, there was no effect on erythrocyte or brain acetylcholinesterase (AChE) activity. Kidney and liver weights were also increased in mid- and high-dose males and females.

To further assist with selecting the highest dietary level, a pilot study was performed with Wistar rats that were treated from GD 6 through day 21 of lactation I postnatal development, with dietary levels of 0, 200, 400 and 600 ppm, with adjustments during lactation to maintain a more constant dosage throughout exposure. [8]. Excessive toxicity was evident at 600 ppm, including convulsions and decreased body weight in the dams (-14% on GD 20) and offspring (-18% on PND4). More moderate toxicity was evident at 400 ppm, with no clinical signs of toxicity and decreased body weight in the dams (-11% on GD 20) and pups (-11% on PND 11). The effects on the dam and offspring were slightly less at 200 ppm. The Health Effects Division (HED) does not think 600 ppm shows excessive toxicity. In addition data was not provided as to how many animals exhibit convulsions.

Based on these combined results, the dietary levels for the developmental neurotoxicity study were 0, 50, 150 and 400 ppm, with adjustments during lactation to maintain a more constant dosage throughout exposure. The 400 ppm dietary level was selected as a maximum dose the animals will tolerate without excessive toxicity. The 150 ppm dietary level was selected as an intermediate dose that may produce effects that can be compared to the reproduction study and to assist in establishing compound-related effects. Finally, the 50 ppm dietary level was selected to establish an overall NOAEL in the offspring, with minimal or no effects evident in the dam.

6. Dosage administration: The treated feed was provided to the dams for consumption beginning on GD 6 and continuing for the dams and offspring (as they were weaned) through lactation day 21.

7. Dosage preparation and analysis: Concentrations of the test substance in the diet were measured by GC/MS [3], using five batches of feed that were used in this study. The stability (at room temperature and freezer conditions) and homogeneity of the test substance in the feed were originally verified at dietary levels of 40 and 600 ppm [4]. See Attachment 1, page 776. While this study was in progress, an additional lower dietary level of 18 ppm was verified in order to bracket dietary levels used in this study, due to adjustments during lactation.

Four dose groups (approximately 30 females/dietary level) were administered the test substance in the diet at nominal concentrations of 0, 50, 150 or 400 ppm. Formulations were prepared weekly by mixing appropriate amounts of the test substance in the diet Purina Mills Certified Rodent Diet 5002 in meal form and were stored at freezer (-5.0 to -26.7°C) conditions. Dietary concentrations were not adjusted to correct for purity (percent active ingredient) in the test substance but were adjusted (reduced) during lactation, relative to gestation, to maintain a more constant level of exposure (mg/kg/day). After day 21 of postnatal development, untreated feed was provided for consumption to all groups. A given batch of feed was available for *ad libitum* consumption for a period of one (GD 0- LD 21) or two (post-weaning) weeks prior to changing, at which time any uneaten feed was collected and disposed of by incineration.

Prior to the start of the study, stability of the test substance in the diet was originally evaluated for a period of 14 days at room temperature (18-26°C) and 182 days at freezer (-20°C) conditions at dietary concentrations of 40 and 600 ppm. Also during the study, stability of the test substance in the diet was evaluated for a period of 7 days at room temperature (18-26°C) and 14 days at freezer (-20°C) conditions at a dietary concentration of 18 ppm. Homogeneity (top, middle and bottom) was originally evaluated once (prior to initiation of this study), at dietary concentrations of 40 and 600 ppm and during the study at a dietary concentration of 18 ppm During the study, samples of each batch of feed were analyzed to establish dietary concentrations (see Attachment 1, page 776).

Results –

Homogeneity Analysis: Homogeneity of the test substance in the ration was accepted for concentrations that bracketed those used in this study. These concentrations of 18, 40 and 600 ppm had percent relative standard deviations (%RSD) of 4.5%, 1.2% and 6.1%, respectively [4].

Stability Analysis: The stability of endosulfan in the ration was established at room temperature for dietary concentrations of 18, 40 and 600 ppm, with no appreciable decrease in concentration with 7 (18 ppm) or 14 (40 and 600 ppm) days of storage. Endosulfan was also stable at freezer conditions for 28 (18 ppm) or 182 (40 and 600 ppm) days, with no appreciable decrease in concentration [4].

Concentration Analysis: For gestation, the nominal 50, 150 and 400 ppm dietary levels averaged 97%, 95% and 96% of the nominal concentrations, respectively. Based on these results, the average dietary levels during gestation for this study were 0, 48.3, 142 and 386 ppm, respectively. For lactation, dietary levels were adjusted to achieve a more consistent dosage (mg/kg/day) throughout the period of exposure, since food consumption increases during this time period. The nominal dietary levels and analytical results were as follows:

Lactation Week[a]	**1**			**2**			**3**		
Nominal Concentration (ppm)	26	79	211	22	65	174	18	54	143
Mean Concentration (ppm)[1]	23.8	73.4	207	19.6	60.5	172	17.0	52.9	143
% Nominal	92	93	98	89	93	99	94	98	100

[1] Analytically confirmed.
[a] Data obtained from page 24 in the study report MRID 46968301.

8. **Analysis of Feed and Water:** Each lot of feed was analyzed by Purina Mills Inc. Laboratories (St. Louis, MO). Contaminant concentrations outlined in the Certification Profile for Purina Mills Certified Rodent Diet 5002 [2] were used as a general standard by which to gauge acceptable levels of contaminants in the feed. Tap water (Kansas City Missouri Municipal Water) was analyzed monthly for contaminants by the city of Kansas City, MO and periodically throughout the year by Pace Inc. (Lenexa, KS).

9. **Test Substance Analysis:** The identity of the active ingredient was confirmed by analytical methods. The concentration of the active ingredient in the test substance was measured within six months of initiation of dietary exposure in order to verify the stability of the test substance at room temperature storage conditions.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals:

1) Clinical Observations: Following acclimation and continuing until animals were removed from the study, P-generation males and females were observed (cage-side) for clinical signs at least once daily. These observations were sufficient to characterize mortality, moribundity, behavioral changes, and overt toxicity by viewing the animal in its cage. At the discretion of the observer, the animal was removed from the cage for a more detailed examination.

2) Detailed Observations: A detailed evaluation of the dams for clinical signs with a physical examination was conducted once daily from the initiation of exposure (GD 6) through lactation day 21. These observations were performed by an individual who was aware of the animal's dosage group assignment.

3) Functional Observational Battery: Animals that were presumed to be pregnant (approximately 30 per dietary level) were observed on GD 13 and GD 20 and a minimum 10 dams/dietary level that were maintained on study with suitable litters were also observed on LD 11 and LD 21. All observations were performed by an individual who was unaware of each animal's dose group assignment. This evaluation was performed under standard animal room conditions (temperature, relative humidity, etc.) and included observations in the home cage, during handling, and outside the home cage in an open field (one minute), using standardized procedures. Since it was not feasible for one person to evaluate all animals on all test occasions, the laboratory maintains evidence of inter-observer reliability (agreement) for individuals who were involved with performing these observations [9,10]. This observational battery included, but was not limited to, assessments (with severity scoring) of lacrimation, salivation, piloerection, exophthalmia, urination, defecation, pupillary function, palpebral closure, convulsions, tremor, abnormal movements, unusual behaviors, posture and gait abnormalities.

FUNCTIONAL OBSERVATIONS
X Signs of autonomic function including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence of absence of piloerection and exophthalamos, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X Description and incidence of posture and gait abnormalities.
X Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, arid any other observations that may facilitate interpretation of the data.

4) Body weight and food consumption: Body weight and food consumption were measured once weekly during gestation and lactation, as follows: Gestation days 6-13, 13-20 and lactation days 0-7, 7-14 and 14-21. In addition, dams were weighed on LD 4. Measures of food consumption may have included consumption by the pups, especially during the third week of lactation. Fresh feed and clean feeders were provided weekly.

5) Delivery and Culling: Each dam was evaluated daily for evidence of delivery from GD 20 to the completion of delivery, which was designated lactation day 0 (LD 0) for the dam and postnatal day 0 (PND 0) for the pups. Litter size (the number of pups delivered) and pup "status" -at birth were recorded for each litter, if a dam delivered fewer than three pups per sex or if the litter size decreased to fewer than seven pups by PND 4, the dam and litter were sacrificed without necropsy examination. For litters that met the minimum size requirements, the size of each litter was adjusted on PND 4 to yield, as closely as possible, four males and four females. Adjustments of litters were made by random selection of the pups using SAS [1] applications, if the number of male or female pups was less than four, a partial adjustment was made (e.g., three females and five males). If there were more than 23 acceptable litters for any dietary level, the surplus litters were sacrificed on PND 4 after weighing without routine necropsy, with preference given to retaining litters with a full complement of four males and four females. Culled dams and pups were sacrificed by CO2 asphyxiation and decapitation, respectively. Dams with insufficient litters were also sacrificed by carbon dioxide (C02) asphyxiation.

6) Moribund Animals and Animals Found Dead: Parental-generation males and females that were found moribund (if any) while on study were sacrificed by CO2 asphyxiation. Dams that were found dead or moribund (if any) underwent a gross necropsy examination, with possible collection of tissues, if the study director determined this was necessary to assist in determining the cause of death. P-generation males that were found dead or moribund (if any) did not undergo a necropsy examination aid were disposed of without routine collection of tissues.

7) Termination: P-generation males and females were sacrificed by CO2 asphyxiation. A gross necropsy examination was not performed routinely on these animals.

Males: Following co-habitation, males were sacrificed by CO2 asphyxiation and discarded unless an alternative use was found.

Females: Dams were sacrificed on LD 21, following the weaning of their respective litters. Females that were sperm positive and/or had an internal vaginal plug, but did not deliver, were generally sacrificed on GD 24 without necropsy examination.

b. Offspring:

1) Litter observations: The day of completion of parturition was designated as lactation day (postnatal day) 0. As soon as possible following parturition, pups were examined for ano-genital distance to establish theft gender, and then were tattooed and weighed. Live pups were counted, sexed and weighed individually for each litter on postnatal days 0,4, 11, 17, and 21. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. More detailed observations for clinical signs were made once daily (a.m.) before weaning and once weekly thereafter. These observations were performed by an individual who was aware of assignments to dose level.

After weaning on PND 21, the remaining pups were weighed once weekly, as well as when vaginal patency or balanopreputial separation was first evident, with detailed observations for clinical signs performed once weekly. Food consumption was not measured after weaning. Fresh feed and clean feeders were provided every two weeks.

2) Developmental landmarks: Beginning on PND 38, male offspring were examined daily for balanopreputial separation. Beginning on PND 29, female offspring were examined daily for vaginal patency. The age of onset was recorded. On PND 21, all pups were also tested for the presence of pupil constriction.

3) Postweaning observations: After weaning on PND 21, offspring were examined twice daily for mortality, and cage-side observations were conducted once daily. Individual offspring body weight data were recorded weekly, as well as on the day that vaginal patency or balanopreputial separation was achieved.

4) Body Weight and Food Consumption: Surviving pups were weighed on PND 0, 4, 11, 17, and 21, and once weekly thereafter. The individual pups were also weighed when vaginal patency or balanopreputial separation were first evident. Food consumption was not measured after weaning on PND 21, when all animals received untreated diet. Fresh feed and clean feeders were provided every two weeks.

5) Neurobehavioral evaluations: Observations and the schedule for those observations are summarized as follows from the report. The test room used for motor activity, auditory startle habituation and passive avoidance conditioning was a standard animal room that was set to be maintained on the same light:dark cycle as the room in which animals were housed, with tests conducted during the light phase. The water maze testing was performed in the room where animals were housed. The order of testing and assignment of animals to specific test devices was semi-random, such that groups were balanced across test times and devices and no animal was tested more than once in the same device. One planned exception was that animals were purposely tested in the same water maze on both occasions, as per standard procedure. Males and females were generally tested on the same days at the appropriate days of age. After sexual maturation, test devices were cleaned during the ensuing interval to reduce the residual scent from the other gender.

I) Functional observational battery (FOB): On postnatal days 4, 11, 21, 35 (± 1 day), 45 (± 1 day), and 60 (± 2 days), approximately 16 offspring/sex/group (minimum one male or one female from each litter) were examined outside the home cage in an FOB assessment, as appropriate for the developmental stage involved. This evaluation was performed according to the procedures described for maternal animals (see above), using standardized procedures. The only difference is that the neonates (i.e., PND 4 and 11) were not evaluated in the open field, since this is routinely done only if the observer considers it necessary for evaluation and this was not the case in the present study.

II) Motor activity testing: Motor activity was measured for approximately16 rats/sex/dose (minimum 10/sex/dietary level) on PND 13, 17, 21 and 60 (± 2 days). The same offspring were evaluated in the figure-eight maze for 60 minutes at each time point, using a computer-automated system (Columbus Instruments, Columbus, OH) and personal computer for automated data collection. The figure-eight maze was selected as an established and widely used automated activity device that can be used to detect both increases and decreases in activity [13]. Each maze consisted of a series of inter-connected alleys (approximately 10 x 10 cm in cross-section) converging on a central arena and covered by transparent acrylic plastic [14]. Each maze had eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys) to measure activity and an activity count was registered each time a beam was interrupted. The floor of each maze rested above absorbent paper, which was changed routinely at the end of each day. A Columbus Instruments (Columbus, OH) Universal Maze Monitoring System and a personal computer were used for automated data collection. Broad-spectrum background noise [74± 2dB(A)] was provided throughout the test to minimize acoustical variations during testing. The uniformity of light intensity (100 ± 70 Lux) over each maze was verified daily. Motor and locomotor activities were examined as total activity counts (beam interruptions) for the 60-minute session and as activity during each ten-minute interval. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Thus, for locomotor activity, only one interruption of a given beam was counted until the rat relocated in the maze and interrupted a different beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

III) Auditory startle reflex habituation: Auditory startle reflex habituation testing was performed in approximately 16 rats/sex/dose (minimum10 offspring/sex/dose) on postnatal days 22 and 60 (± 2 days), using an automated system. A personal computer was used to control the operation of an integrated startle response test system (Coulbourn Instruments, Allentown, PA) and for automated data collection. Groups of four animals (maximum) were tested simultaneously within each of two startle system enclosures. Each enclosure was ventilated, lined with sound-attenuating and vibration-absorbing material, and houses a speaker mounted in a central position within the ceiling of the enclosure to provide the eliciting stimulus (S2) - a 50- msec burst (0 msec rise/fall) of broad-spectrum "white" noise [approximately 118 dB(lin)]. Each enclosure also houses four load cell/force transducer assemblies that are designed to measure the startle response. During the test session, animals were placed into individual restraining cages that were positioned on top of each load cell. The test session consisted of 50 trials that began following approximately a 5-minute adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus at 10-sec intervals. The peak response amplitude was determined for each trial as described below. The average response amplitude and the magnitude of decrease (habituation)

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over blocks of ten trials were compared among the dosage groups. Data collection began with the presentation of S2 and continued thereafter for 200 msec. The analog signal for each response output (measured in my) was digitized at one kHz (i.e., one sample/msec for 200 msec) and converted to grams using a previously determined calibration curve for each load cell. Peak response amplitude (g) and latency (msec) measurements were taken from each animal's individual response curve [16]. Baseline was defined as the average force (g) exerted on the platform during the first 8 msec following the onset of S2, a time period that precedes response onset [17]. This baseline value was taken to represent an approximate body weight measurement that was used to verify that the equipment used to measure the response amplitude was functioning properly. Response amplitude is defined as the maximum value of the average curve, minus the baseline (i.e., removing the animal's body weight from the measurement). Latency to peak is the time (msec) following the onset of S2 when the peak response amplitude occurs.

IV) <u>Learning and memory testing:</u> Learning and memory testing was performed in approximately 16 rats/sex/dose (minimum10 offspring/sex/dose). The same set of animals was used for testing passive avoidance (on PND 22 and 29) and water maze (PND 60 (±2 days) and again seven days later).

Postweaning - Passive avoidance: Animals were tested for acquisition on PND 22 and for retention on PND 29. Testing was conducted using equipment and computer programs from Coulbourn Instruments (Allentown, PA). A personal computer was used to control the operation of the equipment and for automated data collection. Testing took place in individual isolation cubicles, each housing a single shuttle cage. Each isolation cubicle was lined with foam insulation to attenuate sound in the chamber and had a fan with a baffled air intake and exhaust system for ventilation. The shuttle cage consisted of a Plexiglas and stainless-steel rectangular chamber fitted with front-loading access. Each shuttle cage (15 inches wide x 7.25 inches deep) was separated into two compartments of equal size (approximately 7 x 7 inches) by a wall that supported a centrally-located sliding (guillotine-type) door. The two compartments were identical, except that the walls in one compartment were lined with black film (dark-side) and the walls in the other compartment were not lined and it was illuminated during the test with a high- intensity lamp. The lamp was switched on to illuminate the light compartment at the start of each trial and remained on until either the animal crossed to the dark compartment or the trial ended. The floor of the cage consisted of a grid of stainless-steel bars. The movement of the animal from the starting (light) side to the dark compartment was detected by a photocell system. A Coulbourn solid-state scanning shock generator was used to deliver a brief (0.5 sec) pulse of mild (0.5 mA) distributed shock to the grid floor when the animal crossed to the dark compartment.

The test was conducted according to established procedures [18]. After adaptation, individual animals were placed individually into the "lighted" compartment of a conditioning apparatus (the shuttle cage), facing toward the light. After approximately 60 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the non-illuminated side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered, and the light switched off - signaling the end of that trial. At that time, the animal was returned promptly to the holding cage to wait for the next trial. If the rat failed to cross within 180 sec, it was returned to the holding cage and the latency assigned an arbitrary score of

180. This restriction dictated the use of nonparametric statistical analyses. The procedure was repeated until either the rat remained in the lighted compartment for 180 sec on two consecutive trials or until 15 trials had elapsed, whichever occurred first. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable. The test was repeated one week later. For this second trial, rats were placed in the illuminated side of the apparatus, given a 20-sec acclimation period, and the latency to enter the dark side recorded.

Animals that either failed to reach criterion performance within 15 trials or failed to cross during the first two trials during acquisition were excluded from the retention phase of the experiment. The dependent measures were the number of trials-to-criterion, latency to cross on Trial 1 and Trial 2 (learning phase only) and the number of rats/group that failed to reach criterion within 15 trials (learning phase only).

Adult (PND 60) Offspring - Water maze: Animals were tested on PND 60 (-2 days), and again seven days later. Only animals that demonstrated acquisition were tested for retention. The water in the M-maze was maintained at 22 ±1°C. The mazes were constructed of opaque Plexiglas, with corridors approximately five inches wide and walls approximately 16 inches high with approximately 7.5 inches of water. This maze was selected as an established and widely- used device that can be used to measure associative learning and memory.

On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first (learning) trial, the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this learning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (± 5) seconds. Each rat was required to reach a criterion of five consecutive error- less trials to terminate the test session. The maximum number of trials in any test session was fifteen. Latency (in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial.

Animals that satisfied the above criteria within the 15-trial limit were tested for retention seven days following acquisition (animals that failed to reach criterion during acquisition were excluded from the retention phase of the experiment). The correct goal and the criterion were the same for both sessions. Dosage groups were compared for the following dependent measures:
Measures for acquisition included the number of trials-to-criterion, the average number of errors (incorrect turns in the maze) for each trial and the latency (in seconds) to reach the correct goal on trial 2 (a measure of short-term retention). Measures for retention included the number of trials-to-criterion, the average number of errors for each trial, and the latency (in seconds) to reach the correct goal on trial 1 (a measure of long-term retention).

6) **Ophthalmology:** At approximately 50-60 days of age, ophthalmic exams were conducted using the males and females (a minimum of 10/sex/dietary level; representing at least 20 litters per level) that were selected for perfusion at study termination, If needed to clarify the significance of findings, the animals reserved for adult brain weight measurements were also subjected to

ophthalmologic examination. The exam took place in a semi-darkened room. The pupillary reflex was tested using a penlight or transilluminator, with a mydriatic agent applied to each eye to dilate the pupil. The conjunctiva, cornea and lens were examined with a slit lamp microscope either before or after pupillary dilatation. After mydriasis, the vitreous humor, retina, choroid, and optic disc were examined using an indirect ophthalmoscope equipped with a condensing lens.

7) Postmortem observations:

a. Maternal animals: Maternal animals were sacrificed by CO2 asphyxiation on day 21 of lactation following the weaning of their respective litters. The dams were discarded without postmortem examination. Females that were sperm positive and/or had an internal vaginal plug but did not deliver were sacrificed on GD 24 without necropsy examination.

b. Offspring:

Sperm Analysis: Sperm samples were collected from the left testes and left epididymis for enumeration of homogenization-resistant spermatids and cauda epididymal sperm reserves, respectively at sacrifice for one male from each of the first 10 litters at each dietary level. In addition, an evaluation of the morphology and motility was performed on sperm sampled from the distal portion (closest to the urethra) of the left *vas deferens*. Morphology and counts were conducted on the control and high-dose group only. Sperm motility and counts were conducted using the Integrated Visual Operating System (1VOS, Hamilton-Thorne Research, 1998) [22].

Necropsy: The offspring selected for brain weight or neuropathological evaluations were sacrificed on PND 21 or 75 (± 5 days). Fl-generation animals that were found moribund (if any) while on study were sacrificed and underwent a gross necropsy examination. Tissues were collected at the discretion of the study director. In addition, randomly selected animals from Sets A-C that were used to measure fresh brain weight underwent a necropsy examination. Where required, the necropsy involved an examination of all organs (including the brain), body cavities, cut surfaces, external orifices and surfaces, with all gross abnormalities recorded. Gross lesions in neural tissues or skeletal muscle were appropriately sampled for microscopic examination. Other gross lesions were generally not collected for microscopic examination. Animals found dead (if any) underwent a necropsy examination and were disposed of without the routine collection of tissues.

Perfusion: Animals that were selected for perfusion on PND 21 (from Set D) or at study termination (from Sets A-C) were deeply anesthetized using an intraperitoneal dose of pentobarbital (approximately 50 mg/kg) and then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by *in situ* fixation using universal fixative (1.0% (wlv) glutaraldehyde and 4% (w/v) EM-grade formaldehyde) in phosphate buffer. On PND 21, only the brain (with olfactory bulbs) was collected. At study termination, the brain and spinal cord, both eyes (with optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs and physical identifier were collected. All tissues were post-fixed in 10% buffered formalin. The brain was weighed upon removal from the skull, prior to placement into formalin, and the brain: body weight ratio calculated.

Measurements: Prior to sectioning the brain for histology, a Vernier caliper was used to obtain two linear measurements (mm).

1. Anterior-to-posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and

2. Anterior-to-posterior (AP) length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole.

These gross measurements were performed by an individual who was aware of dose group assignments.

Histology: The brain tissues from perfused animals, and any gross lesions collected at necropsy, were further processed for microscopic examination. After the gross measurements were taken, the brain was divided into eight coronal sections for microscopic examination. The eight brain sections were processed according to standard procedures for paraffin embedding, sectioned at approximately 5 um, and examined after staining with hematoxylin and eosin (H&E). In addition, the brain sections reserved for morphometric measurements (levels 3-5 and 7) were stained using luxol fast blue/cresyl violet. Additional tissues were collected for microscopic examination from animals that were perfused at study termination. This included three levels of the spinal cord (cervical, thoracic and lumbar), the cauda equina, eyes, optic nerves and gastrocnemius muscle were embedded in paraffin and stained with H&E. Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings and gasserian ganglia were embedded in glycol methacrylate (GMA). GMA-embedded tissues were sectioned at 2 j.tm - 3 jim and stained using a modified Lee's stain. Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in GMA resin and sectioned longitudinally. The sciatic nerve was also cut in cross section.

The CHECKED (X) tissues were evaluated for adult offspring.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
	Forebrain		Tibial
	Center of cerebrum		Sciatic
	Midbrain		Sural
	Cerebellum	X	**OTHER**
	Pons		
	Medulla oblongata		
X	**SPINAL CORD**		
	Cervical swelling		Gastrocnemius muscle
	thoracic		Lumbar dorsal root fibers
	Lumbar swelling		Lumbar dorsal root ganglion
X	**OTHER**		Lumbar ventral root fibers
	Gasserian ganglion		Cervical dorsal root ganglion
	Trigeminal nerves		Cervical dorsal root fibers
	Optic nerve		Cervical ventral root fibers
	Eyes		

Micropathology and Morphometry: The tissues from high-dose animals were examined relative to those from the respective control group. If no treatment-related lesion was evident, further analysis was not performed. Any region where treatment-related neuropathology was observed underwent the following semi-quantitative analysis. Sections from all dose groups were coded and examined in randomized order without knowledge of the code. The frequency of each type of lesion was determined with the severity of each lesion graded. The code was then broken and the data evaluated for dose-effect relationships.

Selected brain regions underwent the following quantitative analysis, with the individual performing the measurements aware of dose assignments. Initially, eight linear measurements were taken. If treatment-related effects were evident following this initial evaluation, then additional measurements may have been undertaken. Two of the seven measurements involved gross measurements of the intact brain, as described above. The other five were taken from the histologic sections using software calibrated with an ocular micrometer. These five measurements are described as follows:

1. **Frontal cortex** thickness (Forebrain). This measurement was of the dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm.

2. **Parietal cortex** thickness (Forebrain). This measurement was of the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm.

3. **Caudate putamen** horizontal width (Forebrain; maximum cross-sectional width). This measurement was performed on the coronal section taken at the level of the optic chiasm.

4. **Hippocampal gyrus** thickness (Midbrain). This measurement was of the full width of the hippocampal gnus from the ventral tail of the dentate gnus to the overlying subcortical white matter. Measurements were taken from the hippocampus from both sides of this section, and the mean value was recorded.

5. **Cerebellum height** (Cerebellum / Pons). This measurement extended from the roof of the fourth ventricle to the dorsal surface.

In addition to these measurements, all brain sections from these control and high-dose male and female offspring underwent an extensive micropathologic evaluation.

D. DATA ANALYSIS

1. Statistical analyses: Statistical evaluations were generally performed using software from INSTEM Computer Systems [201, SAS [1] or TASC [21]. The level of significance was set at pł O.05, with the exception of Bartlett's test, which was tested at pł O.OO1. In some cases data may have been visually screened for potential effects prior to being subjected to statistical analysis. In general, continuous data were initially assessed for equality of variance using Bartletñ test. Group means with equal variances were analyzed further using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA. In the event of unequal variances, these data were analyzed using nonparametric statistical procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons). **Functional Observational Battery**. Continuous data were analyzed using an ANOVA, with post-hoc comparisons using Dunnett's test. Categorical data were analyzed using General Linear Modeling and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively [1]. **Motor and locomotor activity** (total session activity and activity for each 10- minute interval) were analyzed using ANOVA procedures. Session activity data for the four test occasions were first analyzed using an ANOVA to determine whether there was a significant day by treatment interaction. For days on which there was a significant treatment effect, Dunnett's test was used to determine whether the treated group was significantly different from the control. Interval data were subjected to a Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by an ANOVA to determine whether there was a significant treatment by interval interaction on each test occasion. For those test days, the data for each interval was subjected to analysis using Dunnett's test to determine whether the treated group was significantly different from the control. **Auditory startle** response amplitude data (peak amplitude) for the three test occasions were first analyzed using an ANOVA procedure. If there was a significant group effect, Dunnett's test was used to determine whether the treated group was significantly different from control. The response amplitude data for each block of ten trials (five blocks/test session) were subjected to a Repeated-Measures ANOVA, using test block

as the repeated measure, If there was a significant group by block interaction, the values for each block were subjected to analysis using Dunnett's test to determine if the results for treated animals were significantly different from control. **Passive avoidance** data were analyzed as follows. Latency data were analyzed using a Wilcoxon Test for time to failure (i.e., time to cross). The number of trials-to-criterion was analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats failing to meet the criterion level of performance in the learning (acquisition) phase was analyzed as incidence data. **Water maze** results were analyzed using parametric and non-parametric tests. Latency data were analyzed by a univariate ANOVA, with post-hoc analysis using Dunnett's test. The number of trials-to-criterion and the number of errors were analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats failing to meet the criterion level of performance in the learning phase was analyzed as incidence data. **Pathology** data were screened for potential effects and then evaluated using the following approach. Additional statistical tests to assess continuous and frequency data may have been used when deemed appropriate.

DATA TYPE	DATA	STATISTICAL TESTS	COMPUTER
	Organ Weight	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)	DATATOX
	Gross Brain Measurements	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)	DATATOX
	Microscopic Brain Measurements	ANOVA and/ or T-test (2)	Corel Quatro Pro
FREQUENCY	Ophthalmology	Visually Screened (3)	Trend.exe
	Gross Pathology	Visually Screened (3)	DATATOX/ Trend.exe
	Micropathology	chi-square Fisher's Exact Test	SAS

All statistical tests used a significance level of $p \leq 0.05$, except for Bartlett's, which used $p \leq 0.01$.

(1) ANOVA was used if data were homogeneous; Kruskal-Wallis was used if data were nonhomogeneous.

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(2) A T-test, 2-tailed, was used for two-group comparisons; an ANOVA followed by a Dunnett's test was used for multiple-group comparisons.
(3) if potential compound effects were suspected, then Chi-Square and one-tailed Fisher's Exact tests were used.

2. Indices:

a. Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:
Mating Index = No, of inseminated females/No, of females co-housed with males X 100 Fertility Index = No. of pregnant females/No. of inseminated females X 100

b. Offspring viability indices: The following viability (survival) indices were calculated from lactation records of litters in the study:
Live Birth Index = No. of live pups born per litter/Total no. of pups per litter X 100 Viability Index = No. of live pups on day 4 pre-culling per litter/No, of live pups born per litter X 100

Lactation Index = No. of live pups on Day 21 per litter / No. of live pups on day 4 post-culling per litter X 100

3. Positive and historical control data: No positive control data were submitted with this study, although several method development/validation studies, in which various neurotoxicants were used, were included in the study report. Positive control data have been submitted to the Agency and are currently under review. Historical control data for FOB, motor activity, startle response, Biel maze and brain morphometry were included with the study report.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No P-generation female deaths occurred during gestation or lactation.

Clinical observations during gestation, lactation and FOB findings could not be definitively attributed to treatment at the MDT and HDT, since the hair loss and rearings not always involved the same dams and did not show a good dose relationship among groups. Four to five females showed hair loss at the two top dose levels during the latter part of gestation. Hair loss was seen in MDT and HDT females for the entire lactation period. Hair loss was also seen in one female in the control and one in female in the lowest dose level. These results are summarized in the following Table 2. Rearings appeared to be nominally increased at the HDT [4.1 in controls and 5.2 at the HDT].

TABLE 2. Maternal clinical/functional observations [a]

Observation	Dose (mg/kg/day)			
	Control	3.74	10.8	29.8
Gestation				
Adult females observed	30	30	30	30
Pregnant females	28	30	28	27
Gestational period (days)	21.6	21.6	21.9	22
No remarkable observations	30	30	25	26
Nasal stain, females affected (gestational period affected) [b]	0	0	0	2 (10-15)
Hail loss, females affected (gestational period effect seen) [b]	0	0	5 (13-21)*	4 (8-21)
FOB, Gestational day 13				
Ease of removal, min. resistance with min. vocalization	9/30	15/30	11/30	16/30
FOB, Gestational day 20				
Ease of removal, min. resistance with min. vocalization	6/30	12/30	9/30	7/30
Lactation				
Adult females observed	23	23	23	23
Not remarkable	23	23	23	21
Nasal stain, females affected (lactational period affected) [b]	1 (15)	0	0	0
Hair loss, number females affected (lactation period effect seen)	1 (21)	1 (0-4)	5 (0-21)*	4 (0-21)
FOB, lactational day 11				
Ease of removal, min. resistance with min. vocalization {N=10}	1/10	0/10	1/10	1/10
Rearing	3.7 ± 1.8	5.2 ± 1.9	6.2* ± 1.8	5.0 ± 1.6
FOB, lactational day 21				
Ease of removal, min. resistance with min. vocalization {N=10}	0/10	0/1-0	1/10	0/10
Rearing	4.1 ± 1.8	4.0 ± 1.6	4.9 ± 1.8	5.2 ± 1.3

[a] Data obtained from pages 66, 68 & 74 and 105-128 in the study report MRID 46968301. [b] One or more animals were affected during these gestational/lactational periods, but all females may not have responded in all days in parentheses * Statistically different ($p < 0.05$) from the control. ** Statistically different ($p < 0.01$) from the control.

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2. **Functional Observation Battery on Dams:** No treatment related effects were seen in dams when tested for FOB for approximately 60 parameters on gestational day 13 and 20 [N=30] and lactational day 11 and 21 [N=10]. Treated groups showed numerically increased effects over control, but the increase was slight and showed no adequate dose relationship. Selected responses, which show nominal increases are shown in Table 2.

3. **Body weight and food consumption:** Selected group mean body weights and food consumption values for pregnant or nursing dams were summarized in Table 3.

Body weight was statistically significantly decreased during gestation at all dose levels. However, the decrease at the lowest dose level appeared to be due to decreased food consumption. It is concluded that the maternal body weight decrement at LDT and possibly at the MDT were due to decreased food consumption and that the decreased in weight of mothers at the HDT was due to toxicity. During gestation body weight [GD 13 and GD 20] and was decreased 5-6% to 14-16% at the LDT to the HDT. This conclusion is supported by the similar food efficiency gestational day 6-13 in control [1.46] and the lowest dose group [1.39] and the nominally decrease efficiency at the MDT [0.51] and the HDT [-1.1]. [Rats frequently show decreased body weight during the first week on a unpalatable diet, but show a more normal food consumption there after.] However during gestational day 13-20 food efficiency at the MDT and HDT was similar or apparently increased over the LDT and control values.

The statistically significant weight loss at the LDT during gestation is attributed to the unpalatable endosulfan in the diet. However, it is less clear that unpalatable test material in the diet caused of the body weight decrement during gestation at the MDT and the HDT. There is the possibility that no toxic body weight decrement was seen in the maternal animals in this study. Especially, when the 90-day neurotoxicity study [MRID# 46444401] is considered. In the latter study no body weight decrement or food efficiency decrease was seen in males or non pregnant females dosed at 45.5 mg/kg/day for 90-days. However endosulfan could be more toxic to pregnant than non-pregnant female rats.

Body weight of maternal animals was statistically decreased at the MDT and HDT during lactational days 0, 4, 7 only, but body weight gain was increased 13% to 78% during lactation at these doses. Food efficiency was unchanged during lactation. The lower body weight seen at the MDT and HDT during lactation was due to failure to regain body weight decrement seen during gestation. Thus the body weight did not decrease further during lactation at any dose level.

Food consumption was decreased at the same dose levels as the body weight decrement during gestation, but not during lactation. Food consumption was decreased 12% to 53% during the first week of the study from the LDT to the HDT and 7% to 17% during the second week of the study at the same dose levels [Table 3]. In contrast to this gestational decreased in food consumption, the decrease in food consumption during lactation was not statistically significant at 5% to 3% from the LDT to the HDT. In addition, food efficiency did not differ from control at any dose level during lactation.

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TABLE 3. Mean (SD) maternal body weight, weight gain and food consumption and food efficiency [a]

Observations/study week	Control	Dose (mg/kg/day)		
	0	3.74	10.8	29.8
Gestation [N = 27-30]				
Mean body weight (g) Gestation day 0	202.5±2.44	196.5±2.71	198.7±2.91	198.5±2.16
Mean body weight (g) Gestation day 6	221.8±3.99	213.9±3.76	220.1±3.16	220.0±2.12
Mean body weight (g) Gestation day 13	250.7±3.16	238.3±3.11*	226.6±3.00*	209.7±2.60**
Mean body weight (g) Gestation day 20	311.6±4.25	293.6±4.24*	282.8±4.11**	268.2±3.36**
Mean body weight gain Gestational days 6-13	28.9	24.4	6.5	-10.3
Mean body weight gain Gestational days 13-20-	60.9	55.3	56.2	58.5
Mean weight gain (g) Gestation days 6-20	89.8	79.7	62.7	48.7
Mean weight gain (g) Gestation days 0-20	109.1±3.10	97.1±2.69*	84.0±3.14**	69.7±2.52**
Mean food consumption (g/animal/day) Gestation days 6-13	19.8±0.39	17.5±0.54**	12.8±0.31**	9.5±0.32**
Mean food consumption (g/animal/day) Gestation days 13-20	21.2±0.43	19.7±0.55	18.1±0.53**	17.5±0.53**
Efficiency of food utilization [g female body wt change]/[g food consumed]				
[g wt gain day 6-13]/[g food consumed day 6-13]	1.46	1.39	0.51	**-1.08**
[g wt gain day 13-20]/[g food consumed day 13-20]	2.87	2.81	3.10	3.34
[g wt gain day 6-20]/[g food consumption day 6-20]	2.19	2.14	2.03	**1.80**
Lactation [N = 27-30]				
Mean body weight (g) Lactation day 0	241.4±3.74	231.2±3.55	219.1±3.27**	210.7±3.64**
Mean body weight (g) Lactation day 4	253.0±3.61	241.4±3.25	234.0±4.00**	226.0±2.51**
Mean body weight (g) Lactation day 7	262.0±3.62	255.7±2.79	245.3±4.04*	241.6±3.53**
Mean weight gain (g) Lactation days 0-7	20.6	24.5	26.2	30.9
Mean weight gain (g) Lactation days 7-14	15.8	18.2	21.8	22.6
Mean weight gain (g) Lactation days 14-21	-6.8	-9.0	-2.1	-.07
Mean weight gain (g) Lactation days 0-21	29.6	33.7	45.9	52.8
Mean food consumption (g/animal/day) Lactation days 0-7	34.2	32.1	31.4	32.2
Mean food consumption (g/animal/day) Lactation days 7-14	50.6	49.1	48.3	48.8
Mean food consumption (g/animal/day) Lactation days 14-21	61.7	58.5	60.7	60.5
Mean food consumption (g/animal/day) Lactation days 0-21	146.5	139.7	140.4	141.5
Efficiency of food utilization [g female body wt change]/[g food consumed]				
[g wt gain day 0-21]/[g food consumed day 0-21]	0.20	0.24	0.33	0.37
[g wt gain day 0-7]/[g food consumed day 0-7]	0.60	0.76	0.83	0.96
[g wt gain day 7-14]/[g food consumed day 7-14]	0.31	0.37	0.45	0.46
[g wt gain day 14-21]/[g food consumed day 14-21]	-0.11	-0.31	-0.31	-0.01

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[a] Data obtained from pages 38, 70, 72, 76 & 78 in the study report MRID 46968301. * Statistically different from control, p<0.05. ** Statistically different from control, p<0.01.

4. **Test substance intake:** Test material intake was based on maternal food consumption and body weight. The doses are expressed as mean daily mg test substance/kg body weight during the gestation and lactation periods [Table 4]. Note the ppm in the diet were not constant, but were adjusted to the body weight to maintain a nearly constant test material consumption in mg/kg/day. Thus this study can not be compared with studies where ppm are maintained constant in the diet through out the study including lactation.

TABLE 4. Mean maternal test substance intake (mg/kg body weight/day) [a]

Period	Dose (mg/kg/day)		
	3.74	**10.8**	**29.8**
Gestation			
Gestation days 6-13	4.0±0.15	8.3±0.24	16.8±0.669
Gestation days 13-20	4.0±0.11	11.3±0.28	32.4±0.397
Gestation days 6-20	4.0	9.8	24.6
Lactation			
Lactation days 0-7	3.3±0.14	10.5±0.26	32.3±0.49
Lactation days 7-14	3.8±0.07	11.9±±0.17	34.8±0.54
Lactation days 14-21	3.6±0.09	12.0±0.18	32.8±0.49
Lactation days 0-21	3.57	11.5	33.3
Gestation and lactation combined [mean]	3.74	10.8	29.8

[a] Data obtained from pages 80 & 81in the study report MRID 46968301.

5. **Reproductive performance:** Results for the maternal animals are summarized from the report in the following Table 5. No effects where seen the reproductive performance of the animals tested. The duration of gestational did not change at any dose level.

TABLE 5. Reproductive performance [a]

Observation	Dose (mg/kg/day)			
	Control	**3.74**	**10.8**	**29.8**
Number mated	30	30	30	30
Number pregnant	28	30	28	27
Number of litters	23	23	23	21
Intercurrent deaths	0	0	0	0
Mean ±SE) gestation duration (days)	21.6±0.10	21.6±0.10	21.9±0.09	22±0.10
Incidence of dystocia	0	0	0	0

[a] Data obtained from page 83 in the study report MRID 46968301. * Statistically different from control, p<0.05. ** Statistically different from control, p<0.01.

6. **Maternal postmortem results:** No gross or microscopic examination of maternal animals was conducted.

B. **OFFSPRING:**

1. **Viability and clinical signs:** Litter size and viability *(survival)* results from pups during lactation are summarized from the report in the following Table 6.

Clinical observations on day 0-21 offspring included, 1-2 pups showing non-treatment related scratches, or bruising on face, back or head from various dose levels. No dose related effects or

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effects that could be attributed to treatment were seen.

Clinical observations on post-weaning rats included, 1-3 animals showing lesions/scabs on the neck or shoulders in control and dosed groups. One male was found dead on day 35 in the control group and 2 males at the LDT on day 35. One female was found dead in the control group on day 30 and one at the LDT on day 41. Urine stains were seen 3 males and 2 females from day 30 – 65 in controls, LDT and MDT. No urine stains were found at the HDT. No dose or treatment related effects were seen in the clinical observations.

TABLE 6. Litter size and viability [a]

Observation	Dose (mg/kg/day)			
	Control	3.74	10.8	29.8
Number of litters	23	23	23	21
Total number born	271	244	255	229
No. pups missing	1	1	3	3
Litters with pups missing	1	1	2	3
No. of pups found dead	4	3	1	0
Litters with pups found dead	4	3	1	0
Mean litter size	11.8	10.6	11.1	10.9
Range in litter size	9-14	8-14	8-13	8-15
Number born dead	0	0	0	1
Sex Ratio Day 0 (% %)	NA	NA	NA	NA
# Deaths Days 0-4 (%)	0	0	0	0
# Deaths Days 4-21 (%)	0	0	0	0
Mean litter size:				
Day 0	12	11	11	11
Day 4 [b]	12	11	11	11
Day 4 [c]	8	8	8	8
Day 11	8	8	8	8
Day 17	8	8	8	8
Day 21	8	8	8	8
Live birth index	100%	100%	100%	100%
Viability index	99.7%	99.5%	99.3%	100%
Lactation index	100%	100%	100%	100%

[a] Data obtained from page 41in the study report MRID 46968301. [b] Before standardization (culling). [c] After standardization (culling).
* Statistically different from control, p<0.05. ** Statistically different from control, p<0.01

2. **Pre-weaning Body weight**: Selected mean preweaning pup body weight data on a litter basis are presented in Table. 7. Offspring body weights [Table 7] during lactation were decreased at all dose levels in males and females on post-natal day (PND) 11 [8% (p<0.01), 11%-12% (p<0.01) and 12%-14% (p<0.01) at the LDT, MDT and HDT, respectively] and in males on PND 17 [7% (p<0.05), 9% (p<0.01) and 11% (p<0.01), respectively] on PND 17 and on PND 21 females at all dose levels [6%, p<0.05, 7%, p>0.05 and 10%, p<0.01, respectively. Offspring weight change was decreased at all dose levels from PND 4-11 in males and females. After day 11, body weight did not change significantly from day 11 to 21. Thus it would appear the pup body weight change during Day 4 to 11 recovered in the LDT group, but failed to recover at the MDT and HDT groups from PND 11 through PND 21 in males and females and in males to PND 70 [Table 8] and in females at the HDT to PND 49 [Table 8].

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TABLE 7. Mean [SE] pre-weaning pup body weights on a litter basis (g) [a]

Postnatal day	Dose (mg/kg/day)							
	Control	3.74	10.8	29.8	Control	3.74	10.8	29.8
	Males				**Females**			
# litters	23	23	23	21	23	23	23	21
0	5.8[0.09]	5.8[0.11]	5.9[0.09]	5.9[0.12]	5.5[0.08]	5.5[0.10]	5.6[0.08]	5.6[0.10]
4 b	9.2[0.18]	9.1[0.21]	8.7[0.19]	8.5[0.25]	8.9[0.17]	8.8[0.17]	8.4[0.17]	8.2[0.24]*
4 c	9.3[0.18]	9.1[0.21]	8.8[0.18]	8.5[0.26]	8.9[0.17]	8.7[0.17]	8.5[0.18]	8.2[0.23]
11	24.3 [0.42]	**22.3** [0.49]**	**21.5** [0.50]**	**21.1** [0.52]**	23.6 [0.36]	**21.7** [0.46]**	**20.9** [0.54]**	**20.4** [0.48]**
17	37.6 [0.67]	**35.0* [0.82]**	**34.3** [0.68]**	**33.3** [0.61]**	36.5 [0.63]	34.1 [0.78]	**33.5** [0.70]**	**32.5** [0.59]**
21	47.5 [0.78]	44.5 [1.10]	**43.9** [0.81]**	**42.5** [0.86]**	45.9 [0.62]	**43.0* [97]**	**42.7* [0.90]**	**41.3** [0.83]**
	Pup weight change [g]							
Day 0-4	3.4 [0.12]	3.3 [0.13]	2.9** [0.12]	2.6** [0.17]	3.4 [0.11]	3.2 [0.11]	2.8** [0.12]	2.6** [0.16]
Day 4-11	15 1 [0.31]	**13.2** [0.38]**	**12.7** [0.36]**	**12.6** [0.33]**	14.7 [0.28]	**13.0** [0.38]**	**12.4** [0.39]**	**12.2** [0.32]**
Day4-17	28.4 [0.60]	**25.9* [0.74]**	**25.5** [0.56]**	**24.8** [0.44]**	27.6 [0.60]	25.4 [0.73]	**25.0** [0.56]**	**24.3** [0.45]**
Day 4-21	38 3 [0.68]	35.4 [0.98]	35.1** [0.68]	34.0** [0.70]	37.0 [0.55]	34.3* [0.90]	34.2* [0.75]	33.1** [0.70]
Day 11-21	23 2 [0.50]	22.2 [0.72]	22.4 [0.47]	21.5 [0.53]	22.3 [0.39]	21.3 [0.67	21.7 [0.50]	20.8 [0.54]

[a] Data obtained from pages 92-93 and 94-95 in the study report MRID 46968301. [b] Before standardization (culling). [c] After standardization (culling). * Statistically different from control, p<0.05. ** Statistically different from control, p<0.01

3. **Post-weaning body weights**: Selected mean post-weaning offspring body weight data are presented in the following Table 8

Offspring post-weaning body weights were significantly decreased at the HDT in males from PND 28 [10%] and day 70 [7%] and females from PND 28 [11%] and day 49 [6%]. Male body weight with decreased at the MDT from PND 35 [11%] and day 70 [6%]. It would appear that the body weight of post-natal offspring continued to recover from pup weight decrement seen during post-natal day 4-11 at the LDT, but not in the MDT and HDT groups.

Table 8: Post-weaning offspring weight[a]

Postnatal Day[b]	Dose (mg/kg/day)							
	Control	**3.74**	**10.8**	**29.8**	**Control**	**3.74**	**10.8**	**29.8**
	Males				**Females**			
28	77.0±10.4 (23)	75.0±7.6 (23)	71.5±6.9 (23)	**69.1*±7.8 (21)**	75.5±10.3 (23)	73.3±6.7 (23)	70.5±6.6 (23)	**67.5*±7.6 (21)**
35	125.4±13 (23)	117.7±113.2 (23)	**111.3*±10.5 (23)**	**110.1*±11.7 (21)**	111.7±9.8 (23)	108.5±8.3 (23)	105.7±7.7 (23)	**102.2*±9.0 (21)**
42	171.6±14.4 (23)	162.2±15.7 (23)	**154.7±*12.8 (23)**	**154.0*±14.6 (21)**	136.8±9.4 (23)	134.6±8.7 (23)	130.8±7.2 (23)	**126.6*±9.8 (21)**
49	214±15.6 (23)	203±17.6 (23)	**194.5*±14.2 (23)**	**193.2*±18.1 (21)**	152.1±9.9 (23)	149.1±9.7 (23)	146,0±8.4 (23)	**142.6*±11.2 (21)**
56	257±17.9 (23)	245.7±20 (23)	**236.9*±16.6 (23)**	**234.9*±21 (21)**	171.3±11.6 (23)	167.2±11.5 (23)	166,4±8.9 (23)	161.9±12.5 (21)
63	289.3±19.3 (23)	277.8±24 (23)	**269.5*±17 (23)**	**267.2*±23.2 (21)**	181.8±11.5 (23)	178.2±11.5 (23)	178,0±9.4 (23)	172.9±12.9 (21)
70	317.6±22.7 (23)	304.8±26.7 (23)	**297.0*±19.1 (23)**	**294.0*±25.1 (21)**	191.0±11.4 (23)	187.6±11.4 (23)	188.2±10.2 (23)	182.9±13,7 (21)

[a] Data obtained from pages 102-103 in the study report MRID 46968301. Values are mean ± standard deviation Actual days of measurements occurred within the week of PND 28,35,42,49,56,63,70
* Statistically different from control, p≤ 0.05
() number of litter

3. Developmental landmarks:

a. **Sexual maturation**: Days to sexual maturation [preputial separation] was statistically significantly increased in males at the MDT [2.2 days, 5%] and HDT [1.9 days, 4%] dose levels. Vaginal opening in females was delayed 1 day at the LDT and 1.2 days at the MDT and nominally [1 day] at the HDT. However, these values for vaginal opening were within the historical control range of 32.0 to 34.6 days. These effects suggest delays in sex maturation during lactation in both males and females. However, the delayed preputial separation was not strictly dose related and vaginal opening was within the historical control range. The data are presented in the following Table 9.

b. **Physical landmarks**: Eye opening and incisor eruption were not reported for males or females. Pupil constriction was measured in days to the criterion in females [Table 9], which were identical for dose groups and controls.

TABLE 9. Mean (SD) age of sexual maturation (days) [a]				
Parameter	Dose (mg/kg/day)			
	Control	3.74	10.8	29.8
Number (M/F)	66/77	67/69	69/69	63/63
Male body wt at criterion	191±2.4	183±2.1	190±3.2	187±2.6
Preputial separation (males)	44.9±0.40	44.8±0.29	47.1*±0.49	46.8*±0.43
Perputial separation historical control range was not presented				
Female body wt at criterion	100±1.7	102±1.5	100±1.6	96±1.7
Vaginal opening (females)	33.0±0.27	34.0*±0.30	34.2*±0.40	34.0±0.40
Vaginal opening historical control range was day 32.0 to 34.6.				
Pupil constriction	21.0	21.0	21.0	21.0

[a] Data obtained from page 45 in the study report MRID 46968301. * Statistically different from control, p<0.05. ** Statistically different from control, p<0.01

4. Behavioral assessments:

a. **Functional observational battery:** Other than a statistically significant treatment related rearing in males at the HDT on PND 45, no other treatment or dose related effects could be ascribed to treatment at any age or in any group [Table 10] and these values were within the historical control range. Rearing was nominally increased over control values at all dose levels for the PND 21 for females and PND 45 for males. Rearing in females was nominally elevated over controls on PND 21 [LDT 5% to HDT 31%, and MDT 52%, p<0.05]. However, only PND 45 males showed a dose related response, which showed statistical significance at the HDT [21% at LDT and 44% at HDT, p<0.05]. PND 21 female showed a marginal dose relationship with statistically significant effects only at the MDT. Data is shown in Table 10 as "Open field rearing means±SD." Since these effects were within historical control range of PND 45 males and PND 21 females the study author's did not believe it was treatment related [historical controls, PND 45 males: 2.9-5.1; PND 21 females: 4.1-6.4]. It is not clear that there is a relationship to treatment for rearing in males or females.

Resistance to removal [minimal] with minimal vocalization was found in PND 4 pups, 3/16 HDT and 1/16 MDT males and 2/16 HDT females and 1/16 females at the LDT. Measurements for PND 11 females 1/16 HDT and 1/16 LDT. The effect was found in only 1/16 control male at PND 35 and 60 [data not shown]. These effects were not dose related, but the relationship to treatment is not clear at these dose levels, but significant numbers of affected animals might be seen at higher dose levels.

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TABLE 10. Functional observational battery results (incidence) [a]

Observation	Dose (mg/kg/day)			
	Control	3.74	10.8	29.8
Males				
Ease of removal, min. resistance and vocalization				
-PND4	0/16	0/16	1/16	3/16
-PND 11	0/16	0/16	0/16	0/16
-PND 21	0/16	0/16	0/16	0/16
-PND-35	1/16	0/16	0/16	0/16
-PND45	NE	NE	NE	NE
-PND-60	1/16	0/16	0/16	0/16
Rearing				
-PND4	NA	NA	NA	NA
-PND 11	NA	NA	NA	NA
-PND 21	4.6±2.2	4.8±2.6	6.6±2.9	5.4±2.8
-PND 35	4.9±3.0	3.8±1.9	4.1±1.7	3.4±2.3
-PND-45	3.4±1.2	4.1±2.2	4.4±1.1	4.9*±1.4
-PND-60	4.1±2.2	4.4±1.6	4.8±3.4	3.9±2.0
Open field – Rearings, Mean±SD [b]	3.4±1.2	4.1±2.2	4.4±1.1	4.9*±1.4
Females				
Ease of removal, min. resistance and vocalization				
-PND4	0/16	1/16	0/16	2/16
-PND 11	0/16	1/16	0/16	1/16
-PND 21	NE	NE	NE	NE
-PND-35	NE	NE	NE	NE
-PND45	NE	NE	NE	NE
-PND-60	NE	NE	NE	NE
Rearing				
-PND4	NA	NA	NA	NA
-PND 11	NA	NA	NA	NA
-PND 21	4.2±1.8	4.4±2.8	6.4*±2.4	5.5±2.5
-PND 35	3.9±1.7	4.4±1.8	5.2±2.4	3.9±2.0
-PND-45	5.4±2.1	4.9±2.2	5.5±2.2	6.1±1.8
-PND-60	7.2±2.7	7.6±2.2	6.4±2.5	6.4±2.9
Open field – Rearings, Mean±SD [b]	4.2±1.8	4.4±2.8	6.4*±2.4	5.5±2.5

[a] *Data obtained from pages 130-184 in the study report MRID 46968301.* [b] Data obtained from page 46 of the submitted report; chosen from the PND at which maximum rearing increase from control to the HDT. N = 16/sex/dose * Statistically different from control, p<0.05. ** Statistically different from control, p<0.01 NE = No effect seen. NA = No data available/presented.

b. **Motor activity:** Total activity data are presented in the following Table 11. All data on motor activity in males and females were within a standard deviation and did not differ from control at PND 13, 17, 21 or 60 [Table 11]. There was a continuous increase in male and female motor activity from PND 13 to PND60. Male motor activity increased about 4-5 fold between PND 13 and PND 21 in control for LDT, MDT and HDT. Female motor activity increased about 4-6 fold PND 13 and PND 21 in control for LDT, MDT and HDT. Motor activity increased about 2-3 fold in males and females between PND 21 and PND60 in control, LDT, MDT and HDT.

Some habituation was apparent in males on PND 17 and PND 21 in all dose groups. At PND 13, activity was too low in controls and dosed groups to determine habituation [See the Appendix Table A]. Females followed the same pattern as the males [Appendix A, Table B]. In general, the biggest difference in activity in males and females occurred between the first and second interval with other intervals showing no definitive difference from each other [See Appendix, Table A and B].

TABLE 11. Mean (S.D.) motor activity data (total activity counts for session) [a]

Test Day	Dose (mg/kg/day)			
	Control38±	3.74	10.8	29.8
Males [N=15-16]				
PND 13	66±66	58±66	65±65	72±76
PND 17	179±110	167±141	199±121	202±171
PND 21	341±80	223±81	316±111	282±127
PND [60]	559±138	532±150	599±101	621±132
Females [N=15-16]				
PND 13	50±42	69±55	56±45	48±33
PND 17	162±157	166±138	210±105	193±116
PND 21	279±109	262±76	281±88	302±106
PND [60]	678±199	802±199	792±182	865±174

[a] Data obtained from pages 187-188 in the study report MRID 46968301.
* Statistically different from control, p<0.05. ** Statistically different from control, p<0.01

Locomotor activity followed a pattern similar to motor activity, increasing continuously from PND 13 through PND 60 [See Table 12]. No difference was seen between control groups and dose groups on any PND measurement. Habituation was seen in control, LDT, MDT and HDT at PND 17 and 21 but not at PND 13 [See Appendix, Table C and D]. However, activity in all interval groups 2-6 was low, especially in the 3rd to the 6th interval and at PND 13 at all intervals.

TABLE 12. Mean (S.D.) locomotor activity data) [a]

Test Day	Dose (mg/kg/day)			
	Control	3.74	10.8	29.8
Males [N=15-16]				
PND 13	6±6	10±26	4±16	9±13
PND 17	38±28	37±37	51±39	55±53
PND 21	101±31	63±22	91±29	87±45
PND [60]	389±110	368±116	422±80	450±125
Females [N=15-16]				
PND 13	5±6	6±9	5±5	6±8
PND 17	37±38	40±37	54±32	46±35
PND 21	80±39	79±23	89±28	83±22
PND [60]	460±157	526±198	531±147	556±113

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c. **Auditory startle reflex habituation**:

Summary of amplitude and latency for males and females are presented in the following Table 13a. Peak amplitude increased from PND 22 to PND 60 as expected with no change in latency.

Interval peak amplitude and latency data are reproduced males in the Appendix, Table E, and for females in the Appendix, Table F. No dose related adverse effects were seen. Peak amplitude decreased within testing blocks, but latency did not change. Latency did not change within the 5 blocks tested in males or females at PND 22 or PND 60 [Table 13b]. PND 38 was not tested.

For ease of comparison interval latency for males and females at PND22 and PND 60 is presented in Table 13b.

TABLE 13a. Mean (SD) overall (Blocks 1-5) acoustic startle peak amplitude (g) and latency to peak (msec)[a]

Dose (ppm)	Parameter	Males		Females	
		PND 22	PND 60	PND 22	PND 60
Control	Peak Amp.	27±10	179±87	25±14	119±90
	Latency	39±5	39±3	39±3	40±4
3.74	Peak Amp.	23±11	154±73	25±13	103±55
	Latency	39±4	40±2	37±3	40±4
10.8	Peak Amp.	25±9	221±129	22±8	91±55
	Latency	38±4	39±2	37±3	42±3
29.8	Peak Amp.	26±10	153±106	23±8	83±38
	Latency	37±3	40±2	38±4	42±5

[a] Data were obtained from Study Report MRID 46968301, pages 211-212; n=15-16. Percent difference from controls (calculated by reviewers) is presented parenthetically. * Significantly different from controls at p<0.05. NA = Data not submitted or otherwise unavailable.

TABLE 13b. Mean (±SD) interval acoustic startle latency to peak (MSEC) in F_1 male and female rats[a]

Block		Dose (ppm in diet) Control	50 PPM	150 PPM	400 PPM
Males					
PND 22	Block 1	41±7	40±7	40±6	38±4
	Block 2	38±8	39±6	40±9	36±5
	Block 3	37±7	41±6	37±5	37±3
	Block 4	38±6	38±5	36±5	36±4
	Block 5	41±8	38±6	38±5	37±4
	Avg. For Total Session	39±5	39±4	38±4	37±3
	No. Of Animals	16	16	16	16
	Body Weight	52	49	46	46
PND 60	Block 1	40±4	42±3	41±2	41±4
	Block 2	39±3	39±3	40±2	40±4
	Block 3	38±3	39±3	38±3	38±3
	Block 4	38±2	39±2	39±4	40±4
	Block 5	39±5	40±2	38±2	39±4
	Avg. For Total Session	39±3	40±2	39±2	40±2
	No. Of Animals	16	15	16	16
	Body Weight	279	259	262	263
Females					
PND 22	Block 1	40±6	39±4	38±4	39±5
	Block 2	39±5	37±6	36±3	38±5
	Block 3	38±5	36±4	37±4	38±6
	Block 4	38±3	37±5	37±5	38±6
	Block 5	39±5	37±6	37±4	36±5
	Avg. For Total Session	39±3	37±3	37±3	38±4
	No. Of Animals	16	16	16	16
	Body Weight	48	45	46	44
PND 60	Block 1	41±4	41±4	42±4	43±5
	Block 2	41±5	40±4	41±4	42±5
	Block 3	38±5	40±5	41±5	43±6
	Block 4	40±8	38±5	43±7	41±7
	Block 5	40±6	40±6	41±5	40±6
	Avg. For Total Session	40±4	40±4	42±3	42±5
	No. Of Animals	15	16	16	16
	Body Weight	170	169	167	158

a Data obtained from pages 211-217 in the study report.
Values are mean ± standard deviation

d) **Learning and memory testing:** The acquisition and retention data are presented in Table 14 for passive avoidance and Table 15 for the water maze.

1) **Passive Avoidance:** Data from post-weaning Passive Avoidance tests in PND 22 and 29 offspring [Table 14] did not differ among dose groups and control. Acquisition and retention were clearly evident in control males and females. On the first test occasion, acquisition was evident in males and females as a marked increase in the latency to cross for the second trial (an average 164 and 180 sec, respectively), compared to the first trial (an average 34 and 26 see, respectively). Moreover, there were no control females that

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crossed within the 180-sec limit after the first trial. In males, 11 of 16 males did not cross within the 180-sec limit after the first trial, while the remaining 5 males did not cross within the 180-sec limit after trial 2 (3 males), 3 (1 male) or 4 (1 male). Thus, acquisition of the avoidance response (a failure to cross within the 180-sec time limit for a trial) was quickly attained in control females and most of the males. As per standard procedure, animals that either failed to satisfy the criteria used to establish acquisition or that failed to cross during the first two trials (and therefore never received the conditioning stimulus) were not tested for retention.

For acquisition and retention, there was no evidence of a compound-related effect in males or females at any dietary level. Moreover, there were no statistical differences from control at any dietary level in females.

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Table 14: Summary of Passive Avoidance Performance at PND 22 and 29 Offspring [mean±Std Dev.] Table was copied as an imaged from page 52 of the report

Session/Parameter		Dose (ppm in diet)			
		Control	50 PPM	150 PPM	400 PPM
Males					
Session 1 (Learning Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	3.5±0.9	3.3±0.7	3.2±0.5	3.1±0.5
	Latency trial 1 (sec)	34.4±30.7	40.1±34.0	23.6±15.1	25.5±19.3
	Latency trial 2 (sec)	164.2±31.0	***180.0±0.0**	179.9±0.6	***180.0±0.0**
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Session 2 (Retention Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	2.0±0.0	2.2±0.5	2.1±0.3	2.1±0.3
	Latency trial 1 (sec)	180.0±0.0	174.0±24.0	172.6±29.7	170.6±29.5
	Latency trial 2 (sec)	180.0±0.0	176.7±13.3	180.0±0.0	180.0±0.0
Females					
Session 1 (Learning Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	3.0±0.0	3.1±0.3	3.1±0.3	3.8±2.3
	Latency trial 1 (sec)	25.8±21.0	36.2±33.5	52.3±54.8	43.9±52.2
	Latency trial 2 (sec)	180.0±0.0	177.1±11.7	172.4±30.4	178.1±5.1
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Session 2 (Retention Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	2.3±0.8	2.1±0.5	2.2±0.5	2.1±0.3
	Latency trial 1 (sec)	178.9±4.4	180.0±0.0	173.1±27.5	169.9±40.5
	Latency trial 2 (sec)	170.0±39.9	179.0±4.1	172.8±28.9	180.0±0.0

[a] *Data extracted from pages 219-220 of the study report*
Trials to Criterion = Mean # Trials per Group ± S.D.
Latency to Trial 1 = Mean Session 1 duration (seconds) per Group ± S.D.
Latency to Trial 2 = Mean Session 2 duration (seconds) per Group ± S.D.
Failed to Meet Criterion = Number of Animals that received the shock but did not demonstrate acquisition.
Failed to Cross = Number of Animals that never received the shock.
** Statistically different from control, p≤0.05*

2) **Water Maze:** In the water maze tests [Table 15], there were no differences among dose

groups and control during the learning phases or the retention phases of the tests. No difference in dosed groups and controls were seen in latency phase. The number of errors was similar among groups of males and females in the learning and retention phases.

Table 15: Summary of Water Maze Performance in PND 60 [±2 days] and 67 [±2 days] Offspring [mean ± Std Dev.] Table copied as an image from page 54 of the report.

Session/Parameter		Dose (ppm in diet)			
		Control	50 PPM	150 PPM	400 PPM
Males					
Session 1 (Learning Phase)	Number of Animals	16	16	16	16
	Trials to Criterion (Mean±S.D.)	7.8±2.7	7.1±1.9	8.5±2.7	7.2±2.7
	Trial 1 - Errors (Mean±S.D.)	1.1±0.8	1.0±1.0	0.8±0.7	1.0±1.0
	Trial 1 - Duration (sec) (Mean±S.D.)	18.1±10.0	20.2±18.0	17.3±10.0	20.2±18.2
	Trial 2 - Errors (Mean±S.D.)	0.7±0.9	0.4±0.7	0.9±1.1	0.4±0.6
	Trial 2 - Duration (sec) (Mean±S.D.)	15.2±10.4	12.1±13.7	17.5±18.2	13.3±10.8
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	1 (6%)
Session 2 (Retention Phase)	Number of Animals	16	16	16	15
	Trials to Criterion (Mean±S.D.)	5.4±0.6	5.8±1.4	5.4±0.8	5.8±1.4
	Trial 1 - Errors (Mean±S.D.)	0.3±0.5	0.7±1.2	0.1±0.5	0.1±0.4
	Trial 1 - Duration (sec) (Mean±S.D.)	10.3±5.2	15.3±15.4	7.6±5.7	9.2±8.8
	Trial 2 - Errors (Mean±S.D.)	0.1±0.3	0.0±0.0	0.3±.0.7	0.1±0.3
	Trial 2 - Duration (sec) (Mean±S.D.)	4.8±3.0	4.1±1.9	6.7±8.4	4.9±3.6
Females					
Session 1 (Learning Phase)	Number of Animals	16	16	16	16
	Trials to Criterion (Mean±S.D.)	6.7±2.2	8.1±3.2	8.5±3.1	7.8±1.9
	Trial 1 - Errors (Mean±S.D.)	0.8±0.9	0.8±1.0	1.4±1.0	1.0±0.8
	Trial 1 - Duration (sec) (Mean±S.D.)	17.7±13.0	16.4±10.6	27.3±20.7	19.1±13.8
	Trial 2 - Errors (Mean±S.D.)	0.6±0.7	0.8±0.8	0.6±0.6	0.9±0.9
	Trial 2 - Duration (sec) (Mean±S.D.)	11.0±6.9	15.6±8.9	15.9±10.4	15.9±9.5
	Failed to Meet Criterion	0 (0%)	1 (6%)	0 (0%)	0 (0%)
Session 2 (Retention Phase)	Number of Animals	16	15	16	16
	Trials to Criterion (Mean±S.D.)	8.9±3.2	7.3±3.3	6.5±2.7	7.2±3.4
	Trial 1 - Errors (Mean±S.D.)	0.4±0.5	0.3±0.6	0.4±0.7	0.5±0.7
	Trial 1 - Duration (sec) (Mean±S.D.)	8.8±5.5	8.9±4.7	10.7±11.3	13.7±12.0
	Trial 2 - Errors (Mean±S.D.)	0.3±0.8	0.0±0.0	0.3±0.8	0.1±0.3
	Trial 2 - Duration (sec) (Mean±S.D.)	6.2±9.4	3.7±0.6	6.4±7.1	5.2±3.1

[a] *Data obtained from pages 222-223 in the study report.*
Values for rats who failed to learn during session 1 were not included in means for session 2.
Values are mean ± standard deviation

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5. **Ophthalmology**: No dose related effects were found on ophthalmic examination. Incidental retinal degeneration was seen in males and females [males 1, 0, 0 and 1; females 0, 1, 0 and 0], respectively in control, 50, 150, and 400 ppm groups.

6. **Postmortem results:**

a. **Sperm Analysis:** Sperm analyses conducted with epididymal and testicular sperm [motility, sperm counts and morphology] in the control and high dose groups. No treatment related effects were seen [Table 16]. None of the dosed groups differed from control for any of the parameters tested.

Table 16: Summary of Sperm analyses [% motile and % progressively motile], total epididymal and testicular sperm count and morphology. Table copied as an image from page 55 of the report.

Parameter	Dose (ppm in diet)			
	Control	50 PPM	150 PPM	400 PPM
	Sperm Motility			
Mean % Motility	86.9	87.3	86.6	87.4
Mean % Progressive	63.5	63.0	64.2	66.4
	Sperm Counts			
Epididymis - Sperm/Gram	319.8	--	--	397.2
Testis – Sperm/Gram	96.0	--	--	89.4
	Sperm Morphology			
Normal	195.5	--	--	197.0
Abnormal	3.4	--	--	2.4
Detached	1.1	--	--	0.6

N=10
-- = Not performed

b. **Brain weights:** Mean brain weight data for males and females are presented in Table 17. The report noted a statistically significant decreased fixed perfused brain weight in PND 21 males at the HDT, but not in perfused or non-perfused PND 75 fresh male brain weights. Females showed no brain weight differences from control in 21 PND or 75 PND animals, either perfused or non-perfused

Table 17: Summary of brain weight data. Table image copied from page 57 of the submitted report.

Parameter	Dose (ppm in diet)			
	Control	50 PPM	150 PPM	400 PPM
Males				
PND 21(Perfused)				
Terminal Body Weight (g)	49.2±3.3 (10)	45.6±5.4 (10)	46.6±2.4 (10)	**43.4*±4.5 (10)**
Brain, Fixed (g)	1.403±0.060 (10)	1.398±0.040 (10)	1.413±0.056 (10)	1.331*±0.063 (10)
Brain, Fixed/Body Weight (%)	2.858±0.151 (10)	3.105±0.375 (10)	3.039±0.155 (10)	3.088±0.214 (10)
PND 75 (±5) (Termination - Perfused)				
Terminal Body Weight (g)	311.4±33.9 (10)	307.0±16.7 (10)	304.5±27.3 (10)	302.4±28.1 (10)
Brain, Fixed (g)	1.833±0.070 (10)	1.791±0.056 (10)	1.783±0.083 (10)	1.812±0.122 (10)
Brain, Fixed/Body Weight (%)	0.594±0.057 (10)	0.585±0.037 (10)	0.589±0.050 (10)	0.602±0.052 (10)
PND 75 (±5) (Termination - Non-Perfused)				
Terminal Body Weight (g)	317.1±25.0 (10)	304.5±19.2 (10)	311.6±19.2 (10)	310.9±28.9 (10)
Brain, Fresh (g)	1.910±0.082 (10)	1.860±0.077 (10)	1.909±0.090 (10)	1.891±0.084 (10)
Brain, Fresh/Body Weight (%)	0.606±0.054 (10)	0.613±0.043 (10)	0.614±0.039 (10)	0.612±0.047 (10)
Females				
PND 21(Perfused)				
Terminal Body Weight (g)	46.2±3.1 (10)	43.7±2.7 (10)	**41.7*±5.6 (10)**	**41.5*±3.1 (10)**
Brain, Fixed (g)	1.344±0.048 (10)	1.361±0.021 (10)	1.306±0.072 (10)	1.317±0.031 (10)
Brain, Fixed/Body Weight (%)	2.920±0.118 (10)	3.128±0.201 (10)	3.173±0.365 (10)	3.194±0.282 (10)
PND 75 (±5) (Termination - Perfused)				
Terminal Body Weight (g)	197.5±18.4 (10)	190.8±18.1 (10)	187.4±15.8 (10)	184.2±16.6 (10)
Brain, Fixed (g)	1.691±0.074 (10)	1.722±0.061 (10)	1.665±0.049 (10)	1.669±0.070 (10)
Brain, Fixed/Body Weight (%)	0.861±0.067 (10)	0.908±0.073 (10)	0.894±0.080 (10)	0.913±0.089 (10)
PND 75 (±5) (Termination - Non-Perfused)				
Terminal Body Weight (g)	193.5±16.0 (10)	190.4±8.8 (10)	193.0±15.8 (10)	181.2±17.3 (9)
Brain, Fresh (g)	1.814±0.057 (10)	1.810±0.066 (10)	1.779±0.112 (10)	1.728±0.105 (9)
Brain, Fresh/Body Weight (%)	0.943±0.073 (10)	0.953±0.064 (10)	0.927±0.093 (10)	0.959±0.079 (9)

ª Data obtained from pages 844-845, 847-848, 850-851 in the study report.
** Statistically different from control, p≤0.05*

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c. **Neuropathology:** Examination of the following nervous tissue was performed. Selected nerve tissue potential effects are presented for controls and the HDT [Table 18]. No treatment related effects were noted. [Only control and HDT animals were examined for these parameters.]

Table 18: Selected nervous tissue examined microscopically at termination in adult offspring [a].

	Males [N=10]		Females [N=10]	
Parameter, number affected/number examined [severity level]	**Control**	**29.8**	**Control**	**29.8**
Brain level 3; dilation	0/10	0/10	0/10	1/10 [3]
Brain level 4; dilation	0/10	0/10	0/10	1/10 [3]
Brain level 5; dilation	0/10	0/10	0/10	1/10 [3]
Eyes; displasia	2/10 [1]	0/10	2/10 [2]	1/10 [1]
Left sciatic nerve; degeneration, nerve fiber	0/10	1/10 [1]	1/10 [1]	2/10 [1]
Right sciatic nerve; degeneration, nerve fiber	1/10 [1]	3/10 [1]	2/10 [1]	1/10 [1]
Left tibial nerve; degeneration, nerve fiber	4/10 [1]	0/10	0/10	0/10
Right tibial nerve; degeneration, nerve fiber	0/10	2/10 [1]	1/10 [1]	1/10 [1]
Spinal cord, cauda equina; cyst	1/10 [2]	0/10	0/10	0/10
Spinal cord, lumbar; anomaly	0/10	0/10	0/10	1/10
Spinal nerve root; degeneration, nerve fiber	3/10 [1]	3/10 [1]	2/10 [1]	1/10 [2]
Severity level- 1 [minimal] to 5 [severe]				

a. Data obtained from pages 861 to 864 from study report, MRID 46968301.

1. **Macroscopic examination:** No findings were reported on macroscopic brain measurements in males or females offspring PND 21 or 75 [Table 19 & 20].

2. **Microscopic Brain measurements:** No difference from controls were seen in microscopic morphometric analysis for brains in males [Table 19] A decrease of 10% [control 1.63, HDT 1.47, $p<0.05$] was seen in the hippocampal measurements in PND 21 females [Table 20], which was within historical control range of 1.38- 1.69 mm. The various sections of the brain at the LDT and MDT were not measured; only controls and the HDT were measured.

Table 19: Microscopic Brain measurements in male rats. Table copied as an image from page 59 of the report.

Parameter	Dose (ppm in diet) Control	50 PPM	150 PPM	400 PPM
	Males			
Gross Measurements				
	Day 21			
Ant/Post Cerebrum Length (mm)	13.20±0.42 (10)	13.25±0.27 (10)	13.26±0.36 (10)	12.96±0.59 (10)
Ant/Post Cerebellum (mm)	7.16±0.35 (10)	7.35±0.28 (10)	7.42±0.31 (10)	7.11±0.44 (10)
	PND 75 (±5) (Termination - Perfused)			
Ant/Post Cerebrum Length (mm)	14.52±0.43 (10)	14.60±0.28 (10)	14.46±0.36 (10)	14.31±0.28 (10)
Ant/Post Cerebellum (mm)	7.76±0.32 (10)	7.79±0.29 (10)	7.62±0.34 (10)	7.76±0.41 (10)
Microscopic Measurements				
	PND 21			
Frontal Cortex (mm)	1.6680±0.0047 (10)	--	--	1.6632±0.0086 (10)
Parietal Cortex (mm)	1.8303±0.0105 (10)	--	--	1.826±0.0068 (10)
Caudate Putamen (mm)	2.7941±0.0144 (10)	--	--	2.7578±0.0295 (10)
Hippocampal Gyrus (mm)	1.6618±0.0044 (9)	--	--	1.5829±0.0199 (9)
Cerebellum (mm)	4.7561±0.1033 (10)	--	--	4.9696±0.0313 (10)
	PND 75 (±5) (Termination - Perfused)			
Frontal Cortex (mm)	1.6643±0.0020 (10)	--	--	1.6546±0.0140 (10)
Parietal Cortex (mm)	1.8667±0.0070 (10)	--	--	1.8523±0.0079 (10)
Caudate Putamen (mm)	3.2391±0.0047 (10)	--	--	3.3121±0.0151 (10)
Hippocampal Gyrus (mm)	1.8145±0.0119 (10)	--	--	1.7412±0.0099 (10)
Cerebellum (mm)	4.7763±0.0582 (10)	--	--	4.8528±0.1659 (10)

[a] *Data obtained from pages 853-854 and 856-857 in the study report.* -- = *not evaluated*
Values are mean ± standard deviation * *Statistically different from control, p≤0.05*

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Table 20: Microscopic Brain measurements in female rats. Table copied as an image from page 59 of the report.

Parameter	Dose (ppm in diet) Control	50 PPM	150 PPM	400 PPM
	Females			
Gross Measurements				
	PND 21			
Ant/Post Cerebrum Length (mm)	12.98±0.46 (10)	12.98±0.30 (10)	13.04±0.27 (10)	13.03±0.23 (10)
Ant/Post Cerebellum (mm)	7.20±0.47 (10)	7.44±0.23 (10)	7.20±0.29 (10)	7.53±0.41 (10)
	PND 75 (±5) (Termination - Perfused)			
Ant/Post Cerebrum Length (mm)	14.27±0.40 (10)	14.43±0.42 (10)	14.32±0.27 (10)	14.14±0.41 (10)
Ant/Post Cerebellum (mm)	7.55±0.44 (10)	7.54±0.27 (10)	7.51±0.35 (10)	7.61±0.31 (10)
Microscopic Measurements				
	PND 21			
Frontal Cortex (mm)	1.6909±0.0050 (10)	--	--	1.6435±0.0082 (10)
Parietal Cortex (mm)	1.9141±0.0074	--	--	1.8466±0.0133
Caudate Putamen (mm)	2.8304±0.0210 (10)	--	--	2.7543±0.0391 (10)
Hippocampal Gyrus (mm)	1.6270±0.0039 (10)	--	--	**1.4692±0.0198* (8)**
Cerebellum (mm)	5.0562±0.0570 (10)	--	--	5.0123±0.0244 (10)
	PND 75 (±5) (Termination - Perfused)			
Frontal Cortex (mm)	1.6702±0.0175 (10)	--	--	1.6996±0.0216 (10)
Parietal Cortex (mm)	1.8260±0.0096 (10)	--	--	1.8649±0.0043 (10)
Caudate Putamen (mm)	3.3741±0.0151 (10)	--	--	3.2627±0.0145 (10)
Hippocampal Gyrus (mm)	1.7380±0.0211 (10)	--	--	1.7220±0.0133 (9)
Cerebellum (mm)	4.8575±0.1197 (10)	--	--	5.0255±0.0486 (10)

ª Data obtained from pages 853-854 and 856-857 in the study report. -- = not evaluated
Values are mean ± standard deviation * *Statistically different from control, p≤0.05*

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATORS CONCLUSIONS: The investigators conclusions were copied as an image from page 61 of the submitted report.

Technical-grade endosulfan was administered via the diet from gestation day (GD) 6 through lactation day (LD) 21 to mated female Wistar rats, at nominal concentrations of 0, 50, 150 and 400 ppm. The offspring were evaluated using detailed clinical observations, body weight, food consumption, developmental landmarks for sexual maturation, automated measures of activity (figure-eight maze), auditory startle habituation, learning and memory (passive avoidance and a water maze task), and an ophthalmic examination. Tissues were collected for morphometry (brain) and microscopic examination on PND 21 (brain) and at study termination (brain, an assortment of additional neural tissues, and skeletal muscle). Lastly, sperm analysis was performed on selected control and high-dose males.

In summary, the following observations were noted.

General
The average daily intake of active ingredient by the dams during gestation and lactation was 0, 3.74, 10.8 and 29.8 mg/kg/day. There was no effect on reproduction parameters at any dietary level.

Maternal

Compound-related effects consisted of the following:

50 ppm None (NOAEL)

150 ppm Statistically significant decreases in body weight, weight gain and food consumption during gestation and statistically significant decreases in body weight during lactation.

400 ppm Statistically significant decreases in body weight, weight gain and food consumption during gestation and statistically significant decreases in body weight during lactation.

Offspring

Compound-related effects were limited to the following:

50 ppm None (NOAEL)

150 ppm Statistically significant decreases in pre- (both sexes) and post-weaning (males only) body weight and similar decreases in body weight gain, a delay in balanopreputial separation and significant decrease in PND 21 terminal body weight (perfused females).

400 ppm Significantly reduced pre- and post weaning body weight and similar decreases in body weight gain in both sexes, a delay in balanopreputial separation and significant

decrease in PND 21 terminal body weight in perfused males and females.
Conclusion: Dietary administration of endosulfan to pregnant rats at the highest tolerated dose of 400 ppm from GD 6 through LD 21 does not produce evidence of neurotoxicity in the offspring.

B. REVIEWER COMMENTS:

Endosulfan technical was administered to pregnant Wistar rats from gestational day 6 [GD] through lactational day 21 [LD] at nominal dose levels in the diet of 0, 50 ppm, 150 ppm or 400 ppm [calculated from food consumption of test material as 0, 3.74, 10.8 or 29.8 mg/kg/day].

This reviewer disagrees with the study author conclusion that the pup weight at the LDT was not an adverse effect. Unless the authors can show otherwise, it is concluded all dose levels showed a pup weight decrement in males. The male and female pup weight was not decreased during the high growth phase between birth and PND 4, but it was decreased between PND 4 and PND 11. The author's comment that the pup weight decrement at the LDT was within historical control range was not supported by data. In addition maternal body weight supported by food efficiency data was depressed from toxicity only at the HDT.

C. STUDY DEFICIENCIES: The claim that the pup weight was within historical control data was not supported. Since pup weight decrement was shown during early lactation at dose levels probably not toxic to dams, a measure of endosulfan in the milk supply would have helped in evaluation of the pup weight decrement. No other study deficiencies were noted that were expected to impact the conclusions.

When reporting data values it is important that in the future the registrant report data using Standard Deviations instead of Standard Error.

For litter size and viability, pages 41, 83-84 for study report MRID 46968301, the Sex Ratio on Day 0 was not reported.

The number of animals included in determining the Post-weaning pup body weights (pgs 102-103) was not reported, only the numbers of litters was reported.

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APPENDIX A

Table A: Summary of Male Interval Motor Activity for Day 13, Day 17, Day 21, and Day 60. [Image copied from pages 193-196 of the submitted report]

Bayer CropScience LP - Toxicology

TECHNICAL GRADE ENDOSULFAN
Summary Interval Motor Activity for Male Rats, Postnatal Day 13
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	17 ± 22	16 ± 19	8 ± 13	9 ± 15	9 ± 12	8 ± 11
50 PPM	12 ± 13	14 ± 24	14 ± 21	6 ± 9	5 ± 9	8 ± 16
150 PPM	12 ± 12	12 ± 20	9 ± 14	9 ± 12	11 ± 18	11 ± 21
400 PPM	14 ± 16	19 ± 24	17 ± 17	11 ± 13	6 ± 13	7 ± 14

Bayer CropScience LP - Toxicology

TECHNICAL GRADE ENDOSULFAN
Summary Interval Motor Activity for Male Rats, Postnatal Day 17
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	66 ± 43	40 ± 31	26 ± 28	14 ± 21	16 ± 20	17 ± 25
50 PPM	69 ± 35	26 ± 32	20 ± 27	12 ± 22	23 ± 49	18 ± 27
150 PPM	62 ± 40	44 ± 37	22 ± 19	24 ± 31	27 ± 40	21 ± 27
400 PPM	64 ± 50	36 ± 39	31 ± 27	29 ± 37	22 ± 31	22 ± 28

Bayer CropScience LP - Toxicology

TECHNICAL GRADE ENDOSULFAN
Summary Interval Motor Activity for Male Rats, Postnatal Day 21
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	121 ± 26	61 ± 21	54 ± 25	44 ± 33	32 ± 22	29 ± 31
50 PPM	101 ± 33	43 ± 24	31 ± 18	17 ± 19	19 ± 16	13 ± 17
150 PPM	111 ± 28	59 ± 29	52 ± 18	34 ± 27	25 ± 21	34 ± 27
400 PPM	103 ± 38	51 ± 31	41 ± 27	31 ± 23	31 ± 27	25 ± 25

Bayer CropScience LP - Toxicology

TECHNICAL GRADE ENDOSULFAN
Summary Interval Motor Activity for Male Rats, Postnatal Day 60
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	116 ± 29	99 ± 37	98 ± 36	89 ± 27	84 ± 35	73 ± 19
50 PPM	108 ± 25	87 ± 22	98 ± 31	81 ± 41	77 ± 43	81 ± 34
150 PPM	126 ± 23	108 ± 25	107 ± 28	93 ± 25	87 ± 27	79 ± 25
400 PPM	124 ± 22	102 ± 30	108 ± 34	101 ± 31	104 ± 28	90 ± 24

* Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Postnatal Day 0 - 06/06/2005
Number of Rats/Group: 15/0 PPM 16/50 PPM 16/150 PPM 16/400 PPM

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Table B: Summary of Female Interval Motor Activity for Day 13, Day 17, Day 21, and Day 60. [Image copied from pages 197-200 of the submitted report]

Bayer CropScience LP - Toxicology

TECHNICAL GRADE ENDOSULFAN
Summary Interval Motor Activity for Female Rats, Postnatal Day 13
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	9 ± 10	7 ± 9	10 ± 11	12 ± 13	8 ± 14	4 ± 10
50 PPM	14 ± 16	15 ± 33	11 ± 12	12 ± 14	10 ± 13	7 ± 13
150 PPM	18 ± 17	12 ± 22	7 ± 12	7 ± 12	8 ± 14	4 ± 8
400 PPM	14 ± 16	5 ± 8	11 ± 16	10 ± 14	3 ± 4	5 ± 7

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TECHNICAL GRADE ENDOSULFAN
Summary Interval Motor Activity for Female Rats, Postnatal Day 17
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	59 ± 37	35 ± 36	17 ± 27	11 ± 18	19 ± 39	22 ± 30
50 PPM	52 ± 36	36 ± 32	26 ± 31	16 ± 26	21 ± 27	14 ± 25
150 PPM	63 ± 30	36 ± 26	33 ± 27	24 ± 23	30 ± 31	25 ± 30
400 PPM	61 ± 48	43 ± 29	31 ± 22	21 ± 26	25 ± 37	14 ± 18

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TECHNICAL GRADE ENDOSULFAN
Summary Interval Motor Activity for Female Rate, Postnatal Day 21
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	101 ± 26	62 ± 26	41 ± 27	28 ± 21	25 ± 26	21 ± 21
50 PPM	98 ± 28	48 ± 23	42 ± 24	34 ± 22	24 ± 19	17 ± 26
150 PPM	94 ± 30	60 ± 17	53 ± 41	30 ± 23	25 ± 27	20 ± 23
400 PPM	105 ± 27	51 ± 25	49 ± 22	42 ± 27	30 ± 27	24 ± 30

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TECHNICAL GRADE ENDOSULFAN
Summary Interval Motor Activity for Female Rats, Postnatal Day 60
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	126 ± 27	109 ± 39	107 ± 54	119 ± 43	121 ± 43	96 ± 32
50 PPM	151 ± 43	139 ± 38	146 ± 59	130 ± 60	119 ± 54	117 ± 58
150 PPM	147 ± 31	126 ± 31	141 ± 44	132 ± 46	127 ± 40	120 ± 39
400 PPM	166 ± 45	137 ± 34	150 ± 48	147 ± 40	141 ± 42	124 ± 32

* Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Postnatal Day 0 - 06/06/2005
Number of Rats/Group: 16/0 PPM 15/50 PPM 16/150 PPM 16/400 PPM

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Table C: Summary of Male Interval Locomotor Activity for Day 13, Day 17, Day 21, and Day 60. [Image copied from pages 202-209 of the submitted report]

Bayer CropScience LP - Toxicology

TECHNICAL GRADE ENDOSULFAN
Summary Interval Locomotor Activity for Male Rats, Postnatal Day 13
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	2 ± 1	1 ± 1	1 ± 1	1 ± 2	1 ± 2	0 ± 1
50 PPM	2 ± 2	3 ± 10	2 ± 6	1 ± 1	0 ± 1	2 ± 6
150 PPM	2 ± 3	1 ± 2	0 ± 1	0 ± 1	0 ± 1	1 ± 2
400 PPM	2 ± 1	2 ± 3	3 ± 5	2 ± 4	1 ± 2	1 ± 1

Bayer CropScience LP - Toxicology

TECHNICAL GRADE ENDOSULFAN
Summary Interval Locomotor Activity for Male Rats, Postnatal Day 17
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	14 ± 12	7 ± 6	5 ± 5	4 ± 6	4 ± 6	3 ± 6
50 PPM	16 ± 13	6 ± 7	4 ± 6	3 ± 6	6 ± 12	3 ± 5
150 PPM	18 ± 14	12 ± 11	4 ± 5	4 ± 6	6 ± 10	7 ± 10
400 PPM	19 ± 20	9 ± 11	7 ± 7	8 ± 11	7 ± 10	6 ± 8

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TECHNICAL GRADE ENDOSULFAN
Summary Interval Locomotor Activity for Male Rats, Postnatal Day 21
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	40 ± 10	18 ± 5	14 ± 7	11 ± 8	9 ± 8	9 ± 12
50 PPM	32 ± 9	11 ± 7	8 ± 5	5 ± 5	4 ± 4	3 ± 5
150 PPM	37 ± 12	15 ± 8	15 ± 6	9 ± 7	7 ± 7	9 ± 9
400 PPM	36 ± 16	14 ± 10	11 ± 9	9 ± 7	9 ± 7	7 ± 7

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TECHNICAL GRADE ENDOSULFAN
Summary Interval Locomotor Activity for Male Rats, Postnatal Day 60
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	84 ± 28	68 ± 32	71 ± 28	62 ± 22	58 ± 24	47 ± 18
50 PPM	77 ± 19	58 ± 21	70 ± 37	56 ± 33	53 ± 34	54 ± 25
150 PPM	90 ± 16	75 ± 22	76 ± 23	63 ± 20	61 ± 24	57 ± 23
400 PPM	91 ± 24	73 ± 28	90 ± 32	72 ± 26	72 ± 25	62 ± 22

* Significantly different from control (p≤0.05, ANOVA)
Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Postnatal Day 0 = 06/06/2005
Number of Rats/Group: 15/0 PPM 16/50 PPM 16/150 PPM 16/400 PPM

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Table D: Summary of Female Interval Locomotor Activity for Day 13, Day 17, Day 21, and Day 60. [Image copied from pages 202-209 of the submitted report]

Bayer CropScience LP - Toxicology

TECHNICAL GRADE ENDOSULFAN
Summary Interval Locomotor Activity for Female Rats, Postnatal Day 13
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	2 ± 2	1 ± 2	1 ± 2	1 ± 2	1 ± 2	0 ± 1
50 PPM	2 ± 1	0 ± 0	1 ± 1	1 ± 2	1 ± 3	1 ± 3
150 PPM	1 ± 1	1 ± 2	1 ± 1	1 ± 2	1 ± 1	1 ± 2
400 PPM	4 ± 5	0 ± 1	1 ± 2	1 ± 1	0 ± 1	1 ± 1

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TECHNICAL GRADE ENDOSULFAN
Summary Interval Locomotor Activity for Female Rats, Postnatal Day 17
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	14 ± 13	8 ± 11	3 ± 6	2 ± 3	5 ± 11	5 ± 8
50 PPM	13 ± 12	9 ± 9	5 ± 8	4 ± 8	4 ± 6	4 ± 7
150 PPM	16 ± 10	9 ± 9	9 ± 9	6 ± 7	7 ± 7	6 ± 7
400 PPM	15 ± 13	9 ± 6	8 ± 7	5 ± 7	5 ± 10	4 ± 6

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TECHNICAL GRADE ENDOSULFAN
Summary Interval Locomotor Activity for Female Rats, Postnatal Day 21
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	32 ± 11	17 ± 6	11 ± 8	7 ± 6	9 ± 8	6 ± 7
50 PPM	32 ± 12	13 ± 7	13 ± 7	10 ± 8	7 ± 6	5 ± 7
150 PPM	35 ± 11	18 ± 9	13 ± 7	10 ± 7	8 ± 9	6 ± 6
400 PPM	32 ± 11	13 ± 5	13 ± 5	12 ± 8	7 ± 6	6 ± 9

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TECHNICAL GRADE ENDOSULFAN
Summary Interval Locomotor Activity for Female Rats, Postnatal Day 60
Study Number 05-D72-YF

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	86 ± 21	70 ± 31	73 ± 45	83 ± 38	86 ± 34	64 ± 29
50 PPM	103 ± 32	91 ± 29	97 ± 47	89 ± 44	73 ± 40	73 ± 37
150 PPM	99 ± 20	82 ± 26	97 ± 39	89 ± 38	83 ± 34	80 ± 28
400 PPM	106 ± 22	82 ± 23	96 ± 41	98 ± 30	96 ± 41	78 ± 27

* Significantly different from control (p≤0.05, ANOVA)
Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Postnatal Day 0 = 06/06/2005
Number of Rats/Group: 16/0 PPM 15/50 PPM 16/150 PPM 16/400 PPM

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Table E: Summary of Male Interval Acoustic Startle Response for Blocks 1-5 for Day 22, and Day 60. [Image copied from pages 214-215 of the submitted report]

Bayer CropScience LP - Toxicology

Date: 25AUG05
Time: 13:53:37

Acoustic Startle Response - TECHNICAL GRADE ENDOSULFAN
Peak and Latency to Peak for Male Rats, Postnatal Day 22
Study Number 05-D72-YF

Treatment Group	Number of Animals	Body Weight	Block 1	Block 2	Block 3	Block 4	Block 5
0 PPM	16	52					
Peak Amplitude			33 ± 17	27 ± 12	28 ± 12	23 ± 10	23 ± 9
Latency to Peak			41 ± 7	39 ± 8	37 ± 7	38 ± 6	41 ± 8
50 PPM	16	49					
Peak Amplitude			25 ± 12	23 ± 13	24 ± 13	23 ± 10	20 ± 10
Latency to Peak			40 ± 7	39 ± 6	41 ± 6	38 ± 5	38 ± 6
150 PPM	16	46					
Peak Amplitude			25 ± 9	26 ± 11	25 ± 11	25 ± 10	22 ± 9
Latency to Peak			40 ± 6	40 ± 9	37 ± 5	36 ± 5	38 ± 5
400 PPM	16	46					
Peak Amplitude			29 ± 11	29 ± 13	25 ± 10	24 ± 12	23 ± 10
Latency to Peak			38 ± 4	36 ± 5	37 ± 3	36 ± 4	37 ± 4

Group*Block Interaction: P - 0.3541
*** Significantly different from control (p≤0.05, ANOVA)
Postnatal Day 0 - 06/06/2005
Body Weight (g) is based on the average output of the first 8 msec of all 50 trials for all animals/g
Number of Trials/Block - 10
Peak Amplitude - Mean ± S.D., Measured in Grams
Latency to Peak - Mean ± S.D., Measured in Milliseconds

Bayer CropScience LP - Toxicology

Date: 25AUG05
Time: 13:53:37

Acoustic Startle Response - TECHNICAL GRADE ENDOSULFAN
Peak and Latency to Peak for Male Rats, Postnatal Day 60
Study Number 05-D72-YF

Treatment Group	Number of Animals	Body Weight	Block 1	Block 2	Block 3	Block 4	Block 5
0 PPM	16	279					
Peak Amplitude			209 ± 92	221 ± 123	202 ± 105	147 ± 99	119 ± 64
Latency to Peak			40 ± 4	39 ± 3	38 ± 3	38 ± 2	39 ± 5
50 PPM	15	259					
Peak Amplitude			209 ± 95	196 ± 101	170 ± 113	115 ± 78	87 ± 36
Latency to Peak			42 ± 3	39 ± 3	39 ± 3	39 ± 2	40 ± 2
150 PPM	16	262					
Peak Amplitude			242 ± 118	286 ± 145	233 ± 148	187 ± 139	158 ± 135
Latency to Peak			41 ± 2	40 ± 2	38 ± 3	39 ± 4	39 ± 2
400 PPM	16	263					
Peak Amplitude			183 ± 99	203 ± 142	157 ± 127	128 ± 106	98 ± 83
Latency to Peak			41 ± 4	40 ± 4	38 ± 3	40 ± 4	39 ± 4

Group*Block Interaction: P - 0.7025
*** Significantly different from control (p≤0.05, ANOVA)
Postnatal Day 0 - 06/06/2005
Body Weight (g) is based on the average output of the first 8 msec of all 50 trials for all animals/gr
Number of Trials/Block - 10
Peak Amplitude - Mean ± S.D., Measured in Grams
Latency to Peak - Mean ± S.D., Measured in Milliseconds

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Table F: Summary of Female Interval Acoustic Startle Response for Blocks 1-5 for Day 22, and Day 60. [Image copied from pages 216-217 of the submitted report]

Bayer CropScience LP - Toxicology

Date: 25AUG05
Time: 13:57:34

Acoustic Startle Response - TECHNICAL GRADE ENDOSULFAN
Peak and Latency to Peak for Female Rats, Postnatal Day 22
Study Number 05-D72-YF

Treatment Group	Number of Animals	Body Weight	Block 1	Block 2	Block 3	Block 4	Block 5
0 PPM	16	48					
Peak Amplitude			26 ± 12	27 ± 15	26 ± 16	24 ± 16	21 ± 15
Latency to Peak			40 ± 6	39 ± 5	38 ± 5	38 ± 3	39 ± 5
50 PPM	16	45					
Peak Amplitude			26 ± 11	25 ± 14	28 ± 19	24 ± 15	22 ± 10
Latency to Peak			39 ± 4	37 ± 6	36 ± 4	37 ± 5	37 ± 6
150 PPM	16	46					
Peak Amplitude			26 ± 15	24 ± 9	21 ± 9	20 ± 7	21 ± 8
Latency to Peak			38 ± 4	36 ± 3	37 ± 4	37 ± 5	37 ± 4
400 PPM	16	44					
Peak Amplitude			24 ± 11	23 ± 9	23 ± 10	22 ± 8	20 ± 9
Latency to Peak			39 ± 5	38 ± 5	38 ± 6	38 ± 6	36 ± 5

Group*Block Interaction: P = 0.8472
*** Significantly different from control (p≤0.05, ANOVA)
Postnatal Day 0 = 06/06/2005
Body Weight (g) is based on the average output of the first 8 msec of all 50 trials for all animals/group
Number of Trials/Block = 10
Peak Amplitude = Mean ± S.D., Measured in Grams
Latency to Peak = Mean ± S.D., Measured in Milliseconds

Bayer CropScience LP - Toxicology

Date: 25AUG05
Time: 13:57:34

Acoustic Startle Response - TECHNICAL GRADE ENDOSULFAN
Peak and Latency to Peak for Female Rats, Postnatal Day 60
Study Number 05-D72-YF

Treatment Group	Number of Animals	Body Weight	Block 1	Block 2	Block 3	Block 4	Block 5
0 PPM	15	170					
Peak Amplitude			129 ± 105	151 ± 126	140 ± 109	98 ± 83	76 ± 58
Latency to Peak			41 ± 4	41 ± 5	38 ± 5	40 ± 8	40 ± 6
50 PPM	16	169					
Peak Amplitude			129 ± 81	124 ± 77	107 ± 81	89 ± 52	66 ± 43
Latency to Peak			41 ± 4	40 ± 4	40 ± 5	38 ± 5	40 ± 6
150 PPM	16	167					
Peak Amplitude			101 ± 47	123 ± 81	93 ± 67	73 ± 48	65 ± 55
Latency to Peak			42 ± 4	41 ± 4	41 ± 5	43 ± 7	41 ± 5
400 PPM	16	158					
Peak Amplitude			106 ± 62	108 ± 56	79 ± 42	63 ± 30	58 ± 26
Latency to Peak			43 ± 5	42 ± 5	43 ± 6	41 ± 7	40 ± 6

Group*Block Interaction: P = 0.6213
*** Significantly different from control (p≤0.05, ANOVA)
Postnatal Day 0 = 06/06/2005
Body Weight (g) is based on the average output of the first 8 msec of all 50 trials for all animals/group
Number of Trials/Block = 10
Peak Amplitude = Mean ± S.D., Measured in Grams
Latency to Peak = Mean ± S.D., Measured in Milliseconds

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13544

R142432

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PC Code: 079401

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